A GLOBAL POPULATION GENETIC STUDY OF PANTALA FLAVESCENS

by

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ABSTRACT OF THE DISSERTATION

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Among Odonata, the dragonfly species *Pantala flavescens* is remarkable due to their nearly global distribution and extensive migratory ranges; the largest of any known insect. Capable of migrating across oceans, the potential for high rates of gene flow among geographically distant populations is significant. It has been hypothesized that *P. flavescens* may be a global panmictic population but no sufficient genetic evidence has been collected thus far. Through a population genetic analysis of *P. flavescens* samples from North America, South America, and Asia, the current study aimed to examine the extent at which gene flow is occurring on a global scale and discusses the implications of the genetic patterns we uncovered on population structure and genetic diversity of the species. This was accomplished using PCR-amplified mitochondrial DNA data to reconstruct phylogenetic trees, haplotype networks, and perform molecular variance analyses. Our results suggested high rates of gene flow are occurring among all included geographic regions; providing the first significant evidence that *Pantala flavescens* should be considered a global panmictic population.

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Introduction

Few insects are as capable of long distance travel as Odonata: species such as *Anax junius* and *Pantala flavescens* have been suggested to circumnavigate the globe. With a nearly global distribution, *Pantala flavescens*, commonly known as the "wandering glider" or the "globe skimmer", may be the most widespread of any known dragonfly species (Russell et al., 1998; Hobson et al., 2012). Primarily circumtropical in their distribution (Samways and Osborn, 1998), they can also be found in many temperate areas including the northeastern United States and southern Canada (Garrison et al., 2006) as well as northeastern China (Feng et al., 2006). Although there are no recorded breeding populations in Europe (Garrison et al., 2006), or much of the far northern hemisphere, there are still instances where *P. flavescens* has been found far outside it's normal range; these include sightings as far north as the Baltic sea (Buczyński et al., 2014) as well as remote island locations in Micronesia (Buden, 2010). Although not rare, as *P. flavescens* is one of the most common dragonfly species on the planet, it is still of interest to academics and the public alike, in part due to its remarkable dispersal abilities.

The extensive range of *P. flavescens* provides important insight into what may be considered the true defining characteristic of this species: their migratory behavior. The migration of dragonfly species is well documented and occurs on all continents with the exception of Antarctica (Wikelski et al., 2006). Yet, of the approximately 2,924 known dragonfly species in existence (Schorr and Paulson, 2015), it is estimated that as few as 25-50 of these species are migratory (Russell et al., 1998). Even among such limited company, the distance and scale of *P. flavescens* migrations is unusually broad. With an

enlarged hindwing base to aid in gliding (Garrison et al., 2006; Anderson, 2009; May, 2013), allowing them to travel extraordinary distances, *P. flavescens* has the longest known migration not just of any dragonfly, but of any known insect (Anderson, 2009; Hobson et al., 2012; Chapman et al., 2015). While many are familiar with the migration of the monarch butterfly in North America, which can travel an incredible distance of up to 4,000km in each direction during their multigenerational migration to Mexico and back (Zhan et al., 2011; Chapman et al., 2015), *P. flavescens* has a migration route which can more than double the overall length of the Monarchs' migration. During their documented multigenerational migration route from India to east Africa and back again, swarms of millions of *P. flavescens* can cover a total distance ranging from, or possibly exceeding, 14,000-18,000km (Anderson, 2009; Hobson et al., 2012).

Not only is the sheer scope of this migration remarkable, but it is also the only known transoceanic migration by an insect; *P. flavescens* flies over 3,500km over open waters across the Indian ocean (Samways and Osborn, 1998; Anderson, 2009; May, 2013). This truly unique behavior is a stark contrast to other migratory dragonflies, such as *Anax junius*, which actively avoid flying over open waters (Russell et al., 1998; May, 2013). Requiring freshwater for reproduction, these migrations across bodies of salt water seem like a counter intuitive, overly risky life strategy. However, it has been shown that *P. flavescens* embarks on these migrations following shifting weather fronts at different times of the year to take advantage of seasonal rainfall, exploiting ephemeral, freshwater rain pools in which they reproduce (Anderson, 2009). Their larvae have a remarkably short development time which can be as rapid as 38-65 days (Suhling et al. 2004; Ichikawa and Watanabe, 2014), allowing them to mature before the temporary pools, in

which they develop, dry out. Newly emerged adults then continue along these migratory routes, following seasonal rainfall patterns and reproducing along the way as they complete their leg of the migratory circuit.

Shifting fronts, such as the Inter Tropical Convergence Zone, not only provide essential freshwater pools for breeding, but the associated winds are what allow *P. flavescens* to migrate long distances while flying at altitudes of over 1,000m (Corbet, 1962; Feng et al., 2006; Anderson, 2009). Physical and behavioral adaptations such as the ability to compensate for wind drift (Feng et al., 2006; May, 2013), slope soaring behavior (Gibo, 1981), feeding on aerial plankton and other small insects (Russell and Wilson, 1997; May, 2013), and an enlarged hind wing base (Garrison et al., 2006; Anderson, 2009; May, 2013), all contribute to the process of energy conservation; critical for long distance migratory flights. Despite these adaptations, *P. flavescens* is still largely at the mercy of the winds upon which they rely, resulting in them being blown far off their intended course in some instances. Populations have been documented in locations as remote as Easter Island where a unique population has exhibited both morphological and behavioral adaptations towards being non-migratory (e.g., they crouch low against the substrate rather than lift their tarsae when wind passes over their wings and possess smaller hindwings than continental populations) (Samways and Osborn, 1998).

The distribution and migratory behavior of *Pantala flavescens* present a unique opportunity to ask questions regarding the amount of gene flow that may be occurring on a global scale as well as its influence on both the population structure and genetic diversity of the species. If a significant amount of gene flow is occurring across continents it is possible that *P. flavescens* could be considered a global, panmictic

population. In this study we aim to address these questions through a population genetic analysis using mitochondrial DNA data of geographically distinct populations of *Pantala flavescens* from North America, South America, and Asia (Fig. 6).

Materials and Methods

Samples of *Pantala flavescens* from Japan, Guyana, India, and the United States were acquired for DNA extraction and sequencing from the field and museum collections. Samples from Japan, provided by Frank Suhling and Hiroshi Jinguji, were collected from Saitama, Miyagi, and Tokyo prefectures between July 2013 and July 2014. All Japanese samples were stored individually in ethanol. Samples from Guyana, India, and the Untied States were collected by various members of Jessica Ware's lab, treated with acetone, then dried, and stored individually. Guyana samples were collected from the Demerara-Mahaica region in North East Guyana over a period of dates ranging from July 2011 through August 2013. India samples were collected from the Angul district of Odisha and from Madanapalle in the Chittoor district of Andhra in August 2012. United States samples were collected from Comal County, Texas in August 2013. Additional sequences used in this analysis from India, Korea, Japan, and Canada were sourced from NCBI GenBank and BOLD Systems. Including the sequences sourced from NCBI GenBank and BOLD Systems a total of 49 *Pantala flavescens* COI sequences were analyzed.

DNA Extraction

DNA extraction was performed on leg and thorax tissue of the *Pantala flavescens*

samples using a Qiagen DNeasy Blood and Tissue Kit. All samples were incubated overnight in 180µl of ATL Buffer and 20µl of proteinase K at a temperature of 56°C. For all centrifuge steps a speed of 10,000rpm was used rather than the manufacturer's suggested 8,000rpm speeds due to limitations of the available centrifuge equipment. In the final step of the procedure a 15-minute incubation time at room temperature was used during elution with AE Buffer in place of the outlined 1-minute incubation time. All other steps of the procedure were performed following the manufacturer's protocol.

PCR Amplification

The mitochondrial gene cytochrome c oxidase subunit I (COI) was chosen to amplify the extracted samples; this was done using two sets of COI primers as listed in Table 1. PCR amplification was performed in 25µl reactions with each reaction consisting of 12.5µl of Taq 2x master mix solution, 1µl of each primer (forward and reverse, both diluted to 1x concentration), 5µl of DNA template, and 5.5µl of RNase-free water. Two thermal cycler programs were used, one for each set of primers. The first program, used for LCO and HCO primers, began at 94ºC for 120 seconds followed by 30 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds, 72ºC for 30 seconds, then an additional 30 cycles of 94ºC for 30 seconds, 53ºC for 30 seconds, and 72ºC for 30 seconds, concluding with 72ºC for 60 seconds. The second thermal cycler program, used for primers coi1709 and coi2191, began with 94ºC for 120 seconds followed by 15 cycles of 94ºC for 30 seconds, 46ºC for 30 seconds, 72ºC for 30 seconds, then an additional 30 cycles of 94ºC for 30 seconds, 48ºC for 30 seconds, and 72ºC for 30 seconds, concluding with 72ºC for 60 seconds. The PCR products were then stained with GelRed loading

buffer and visualized on 1% agarose gel run at a constant 110V for 20 minutes. Successfully amplified samples were both purified and sequenced by Macrogen (NYC, NY, USA) for forward and reverse primer sequences. A total of 40 samples were sequenced: 21 from Guyana, 2 from the United States, 2 from India, and 15 from Japan.

Sequence Analysis

For each sample, forward and reverse sequences were assembled into a consensus sequence, or contig, using Geneious software version 8.1.2 (Kearse et al., 2012). When a single base discrepancy was encountered during sequence assembly that position was coded with either a Y (ambiguous pyrimidine), R (ambiguous purine), or in rare cases an S (C or G), W (A or T), or M (A or C). These sequences were then combined with all available *Pantala flavescens* COI gene sequences from both NCBI GenBank and BOLD Systems for analysis. All sequences were initially aligned using ClustalX 2.1 (Larkin et al., 2007), followed by manual alignment in Mesquite v.3.02 (Maddison and Maddison, 2015). *Trithemis festiva* was chosen as the outgroup based on Ware et al (2007) and Pilgrim and von Dohlen (2008).

As determined by jModelTest 2.1.6 (Darriba et al., 2012), the best nucleotide substitution model was found to be TPM2uf+I+G. We thus implemented the GTR+I+G model. The parameters of this model were used for both Bayesian and maximum likelihood analysis. Bayesian analysis was performed using MrBayes v3.2.3 x64 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) running on CIPRES (Miller et al., 2010) to determine a posterior probability distribution. Two Markov chain Monte Carlo (MCMC) runs were performed; each run consisted of 4 chains running over

the course of 10,000,000 generations with sampling occurring every 1,000 generations. Run convergence and a 10% burn value were confirmed using Tracer 1.6 (Rambaut et al., 2014). Likelihood analysis was carried out using GARLI 2.01 (Zwickl, 2006) to reconstruct both a best likelihood tree (Fig. 1) and a 50% majority rule, 1,000 repetition bootstrap consensus tree (Fig. 2). These trees were summarized and posterior probability support was applied using SumTrees 3.3.0 distributed through the DendroPy 3.12.0 package (Sukumaran and Holder, 2010). Both trees were visualized using FigTree v1.4.2 (available at http://tree.bio.ed.ac.uk/software/figtree/).

An analysis of molecular variance was performed using Arlequin 3.5.1.2 (Excoffier and Lischer, 2010) and GenAlEx 6.5 (Peakall and Smouse 2006; Peakall and Smouse, 2012). Haplotype networks (Fig. 3, Fig. 4, Fig. 5) were constructed using DnaSP (Librado and Rozas, 2009), PopART 1.7 (Bandelt et al., 1999; Clement et al., 2002; available at http://popart.otago.ac.nz), and TCS 1.21 (Clement et al., 2000).

Results

The Bayesian tree (Fig. 1) and the GARLI consensus tree (Fig. 2), along with each haplotype network (Fig. 3, Fig. 4, Fig. 5), suggest a global panmictic population with a high incidence of gene flow among populations from different geographic regions. The presence of polytomies in both trees indicate that there is not enough genetic signal in the COI gene to define populations based on their geography alone in this case. The most prominent instance of regional clustering was exhibited by a small North American clade, present with >50% support in both trees, containing only three individuals: U.S.A. 1, U.S.A. 2, and Canada B2. Interestingly, the second Canadian sample (Canada B1) was

most closely related to one of the India samples (India B1) in each tree with >50% clade support in both cases. This observation, along with the other India samples being grouped with individuals from Japan and Guyana, suggest that there is gene flow occurring among these regions.

Both trees showed individuals collected from Guyana grouped not only with other Guyana samples, but with samples from all other countries in polytomies, small clades, and weakly supported larger clades. One small Guyanese only clade was present in both trees, with >70% support in each, and contained individuals Guyana 8 and Guyana 18. These two individuals were found in the same area as one another but were collected one year apart. Samples Guyana 8 and Guyana 18 were also grouped together as a distinct haplotype in the haplotype networks (Fig.3, Fig.4, Fig. 5), lending further support to this clade. The other Guyana only clade to appear in both trees, consisting of individuals Guyana 12 and Guyana 21, fell below 50% support in both trees. One Guyanese sample, Guyana 16, exhibited a distinct haplotype with 54.9% bootstrap support in the GARLI consensus tree, but was grouped with other Guyanese, Japanese, and Indian samples in a large polytomy in the Bayesian tree. The haplotype networks did not distinguish Guyana 16 as having a distinct haplotype in comparison to other individuals.

Similar to the Guyanese distribution mentioned above, the distribution of Japan samples in both trees showed them being grouped not only with other Japan samples, but with a mix of individuals from other regions as well. There were two small Japan only clades that appeared in both trees. The first contained individuals Japan 1 and Japan 6, collected from neighboring prefectures in Japan one year apart from one another. The second Japanese only clade contained individuals Japan 5 and Japan G1. However, both

of these clades fell shy of having >50% bootstrap support in the GARLI consensus tree.

The only clade not yet mentioned to appear in both trees with >50% support contained individuals Guyana 19 and Japan 3. This clade, along with the distribution of all other individuals in both trees (Fig.1, Fig.2) and the haplotype networks (Fig.3, Fig.4, Fig. 5), strongly suggest that gene flow is occurring on a worldwide scale among multiple populations.

Molecular variance analysis in Arlequin returned an FST value of 0.0426 with 95.74% of variation occurring within populations and 4.26% of variation occurring among populations. A second molecular variance analysis in GenAlEx resulted in a PhiPT value of 0.000 with 100% of variation occurring within populations and 0% occurring among populations. Both of these analyses further imply panmixia and high incidence of gene flow.

The samples India G1 and Korea G1, both sourced from NCBI GenBank, stood out as the most distinct haplotypes in every analysis. It is unclear whether the Korea G1 sample is as distinct as it appears, or if the overall quality of the sequence was not as high as the other sequences used in the analysis. The India G1 sample was listed as "cytochrome oxidase subunit 1-like" on NCBI GenBank, which likely accounts for it being far more distinct than the other COI sequences.

Discussion

Considering the migratory capabilities and extensive ranges of *Pantala flavescens,* that this species may exist as a global panmictic population has been considered possible by odonatologists over several decades (Corbet, 1962; Samways and

Osborn, 1998; Anderson, 2009); yet this theory has not been adequately investigated thus far, and we have little genetic evidence for members of this genus. Our current study may present the first significant evidence to suggest that *P. flavescens* should be considered a predominately global panmictic population rather than a series of geographically isolated, distinct populations. Each of our analyses suggest that, given the mitochondrial data collected, gene flow is occurring on a global scale among *P. flavescens* populations from various geographic regions; suggesting panmixia. The remarkable, large scale migrations of *Pantala flavescens* are likely the primary contributing factor to the observed high rates of gene flow and diminished genetic diversity.

Previous studies have shown similar high rates of gene flow in *P. flavescens*, but on smaller spatial scales. Using randomly amplified polymorphic DNA, Christudhas and Mathai (2014) examined genetic diversity among five geographically isolated populations of *P. flavescens* within India. Their analysis suggested low genetic diversity and uncovered a high rate of gene flow, which implies panmixia among populations within India where annual migrations are known to occur (Fraser, 1936). The samples from India in our analysis ranged from geographically distant southern (India 2), eastern (India 1), and western (India B1) India (Fig. 6); our analyses support the findings presented by Christudhas and Mathai (2014), and suggest that the gene flow is occurring on a large scale, spanning continents. Although more data is needed, specifically to expand sampling in Africa and add genetic loci, these two studies suggest that the migrations of *P. flavescens* have led there to be significant gene flow and reduced genetic diversity.

Based on what we know of the species, it is likely that *Pantala flavescens* can be

considered an obligate migrant. Utilizing adaptations such as an enlarged hind wing base (Garrison et al., 2006; Anderson, 2009; May, 2013), which is ideal for gliding while expending minimal amounts of energy, *P. flavescens* can readily take advantage of prevailing, seasonal winds associated with fronts such as the Intertropical Convergence Zone (ITCZ) (Corbet, 1962; Anderson, 2009; Hobson et al., 2012; May, 2013), allowing them to cover extraordinary distances. The ITCZ provides not only the winds necessary to assist in migrations, but the associated rains produce ephemeral freshwater pools that the dragonflies require for reproduction (Feng et al., 2006; Anderson, 2009). A significant investment in migratory capabilities allow *P. flavescens* to follow favorable breeding weather conditions, resulting in the increased potential to reproduce throughout much of the year (Chapman et al., 2015). Perhaps the most well documented migration of *P. flavescens* thus far has been the transoceanic migratory circuit from India to Africa (Fig. 6) and back again, influenced by the seasonal shifting of the ITCZ in the region (Corbet, 1962; Anderson, 2009; Hobson et al., 2012). Isotopic evidence suggests that the multigenerational journey may total over 18,000km with single individuals traveling over 6,000km during the transoceanic trek from northern India to east Africa (Hobson et al., 2012). This migration exemplifies how the long distance dispersal capabilities of *P. flavescens* are largely passive; a key element in explaining the prevalence of global gene flow in our findings.

Documented observations of *P. flavescens* migrating in accordance with seasonal winds in other parts of the world, such as China (Feng et al., 2006), further reinforce the long distance, passive dispersal capabilities which allow them to circumnavigating the globe. While their reliance on strong winds allows them to cover distances far greater

than any other known migratory insect (Russell *et al*., 1998; Hobson *et al.*, 2012), these same winds can also be responsible for carrying them to areas far from their normal migratory range (Samways and Osborn, 1998; May, 2013). With a tendency to migrate in large swarms (Russell et al., 1998; Anderson, 2009), if even a portion of a migratory aggregation were to be consistently carried by winds in a new direction the impact this would have on increasing gene flow between geographic regions is likely to be significant. High rates of gene flow will counteract divergence (Whitlock and Mccauley, 1999), reducing genetic diversity on a large scale while maintaining a panmictic population.

Passive dispersal has undoubtedly contributed to the observed high rate of gene flow, but it may also be the factor that has resulted in the only documented population of *P. flavescens* that represents an exception to the characteristic migration of the species. This population, described by Samways and Osborn (1998), is found on Easter Island in the Southeastern Pacific Ocean and has developed both behavioral and morphological characteristics that indicate they are non-migratory. In addition to being non-natives, they are the only species of dragonfly found on Easter Island and most likely arrived at this remote location as a result of wind-assisted passive dispersal. While some oceanic islands serve as stopping points along a migratory route, such as the case with the Maldives (Anderson, 2009), this non-migratory Easter Island population raises two important questions: how many other populations exist in extreme isolation and can they still be considered part of a global panmictic population? Further genetic analysis of the Easter Island population, as well as any others that may exist, will be required to answer this question definitively. As for now it appears that extreme isolation may be the one factor

that can influence divergence in the species as no other geographic features have proven to be a challenge up to this point.

The only individuals that exhibited a distinct haplotype in our study were the India G1 and Korea G1 samples. The degree to which the India G1 sequence differed from the rest of the sequences is likely explained by it being "similar to cytochrome oxidase subunit 1" whereas all other sequences were strictly COI. Regarding the Korea G1 sequence, as it was sourced from NCBI GenBank, we are unable to speak to the quality of the sample or sequence in this case. Based on the Bayesian tree in Figure 1, Korea G1 could indicate possible divergence and evolution of new haplotype in the region. However, support for this is not as strong in the GARLI tree in Figure 2. Considering the relation of the second Korean sample (Korea G2) to the other individuals in both trees (Fig. 1, Fig. 2) and the haplotype networks (Fig. 3, Fig. 4, Fig. 5), it would seem unlikely that Korea G1 is as unique as it may appear. However, the possibility that it is a distinct haplotype must be considered and cannot be easily dismissed given the available data. Precise data regarding the collection location and date was not available at the time of writing for this sample. An in depth analysis utilizing said data, in comparison with any known migratory populations in the region, could provide further insight into how distinct this sequence may be.

Gene flow is present. As illustrated in our reconstructed phylogenetic trees (Fig. 1, Fig. 2), haplotype networks (Fig. 3, Fig. 4, Fig. 5), and molecular variance analyses, gene flow is occurring on a global scale among geographic regions. The overall lack of regionally discernible genetic structure in our samples may provide valuable insight regarding the consistency and stability of the migratory routes of *P. flavescens*. Strict

adherence to regional migration routes, expansive as they may be, would likely result in a relatively small number of very large populations spread across the globe (May, 2013). If this concept held true we would have expected to see distinct regional clades in our results; instead we find a mix of polytomies and small clades consisting of both Asian and New World individuals.

The North American clade present in both trees (Fig. 1, Fig. 2) containing all individuals from Texas, U.S.A. (U.S.A. 1, U.S.A. 2) and one individual from New Brunswick, Canada (Canada B2) is the primary exception. However, the consistent grouping of the second sample from New Brunswick, Canada (Canada B1) with the Indian sample from Gujarat, India (India B1) suggests that although there may be a North American migration route, North American individuals are breeding with individuals from other continents. Little research has been dedicated to uncovering North American migratory circuits of *P. flavescens* but, based on the aforementioned clade in our study, it is a topic worthy of further exploration. Future work should sample *P. flavescens* from across Canada, the United States, and Mexico, and evaluate how much true gene flow there is between North America and other continents. Indeed, there could be overlapping, smaller migratory routes that connect North American populations to South American populations, with there being isolation by distance. We do not see this here, but that is likely due to our very small number of US and Canadian samples.

Significant support for dedicated South American and Asian clades was lacking in our analyses. The small Guyanese clade, containing individuals Guyana 8 and Guyana 18, and the comparably sized Japanese clade, containing individuals Japan 5 and Japan G1, were the only exceptions with adequate support in both trees (Fig. 1, Fig 2.). These

clades imply that at least some degree of structure may exist in these regions, but the majority of samples from both these countries were consistently grouped with all other regions in the analysis. The significantly supported Japan-Guyana mixed clade, containing individuals Guyana 19 and Japan 3, exemplify that while some degree of structure may exist within these regions there is still a significant amount of gene flow occurring between them; a trend that holds true for all regions analyzed in this study.

A larger data set of *P. flavescen*s samples collected from an even wider geographic range would be the first step in further assessing rates of gene flow and fluctuations in genetic diversity. In addition to increasing the volume of samples, strategic collection at specific locations and dates coinciding with migratory routes may provide valuable information regarding the stability of these migrations. Techniques such as attaching radio transmitters to migrating individuals have been used to study other migratory dragonflies (Wikelski et al., 2006), but considering the scale of *Pantala* migrations it is unlikely to be effective solution for a multitude of reasons. As any direct measurements of dispersal will encompass a significant number of challenges, genetic analyses used in this study, and isotope analyses used by Hobson *et al.* (2012), will likely prove to be the most efficient method for studying the migrations and population structure of *P. flavescens*.

Although the use of the mitochondrial COI gene has proven to be an effective marker for studying divergence within and among species (Hebert et al., 2003), further genetic analyses would help to evaluate any findings uncovered thus far. The rate of evolution exhibited by mitochondrial DNA is quicker than that of nuclear DNA, making it unlikely that additional analyses using nuclear genes in place of mitochondrial genes

would be more successful in uncovering any recently developed population structure in the species. It is still possible that further genetic analyses using rapidly evolving genetic markers, such as additional mitochondrial genes and microsatellites, may uncover existing structure within populations that the use of COI alone was not able to accurately predict. Previous studies on the age of Odonata, which included *Pantala*, support an origin of the species in the mid to early Miocene (e.g., Ware et al., 2008), yet information regarding the biogeographical origins of the species remain unresolved. Genetic analyses with more loci over an even wider geographic range will be required to address this question.

Our results strongly suggest that *Pantala flavescens* exists in a large panmictic population and that there is worldwide gene flow among populations. However, there is still a significant amount of work to be done in terms of studying *Pantala* on a global scale. In addition to examining their population structure, additional studies regarding the potential ecological impacts of their migratory behavior are worthy of further discussion. As for the future of the species, questions concerning the impact that climate change may have on their migrations and range expansion are noteworthy considerations. Importantly, our study is the first to suggest that genes are being shared among individuals across the globe; this lays the groundwork for future studies, but for this extensive taxon sampling needs to be undertaken. Maintaining a global perspective as studies of *Pantala flavescens* progress will prove to be essential in furthering our understanding of these extraordinary "wandering gliders".

References

Anderson, R.C. (2009). Do dragonflies migrate across the western Indian Ocean? Journal of Tropical Ecology. 25(4):347-358.

Bandelt, H., Forster, P., and Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution. 16(1):37-48.

Buczyński, P., Shapoval, A.P., Buczyńska, E. (2014). Pantala flavescens at the coast of the baltic sea (Odonata: Libellulidae). Odonatologica. 43(1-2):3-11.

Buden, D.W. (2010). Pantala flavescens (Insecta: Odonata) rides west winds into ngulu atoll, micronesia: Evidence of seasonality and wind-assisted dispersal. Pacific Science. 64(1):141-143.

Chapman, J.W., Reynolds, D.R., Wilson, K. (2015). Long-range seasonal migration in insects: Mechanisms, evolutionary drivers and ecological consequences. Ecology Letters. 18(3):287-302.

Christudhas, A. and Mathai, M. T. (2014). Genetic variation of a migratory dragonfly characterized with random DNA markers. Journal of Entomology and Zoology Studies. 2(2):182-184.

Clement M., Posada, D., and Crandall, K. (2000). TCS: a computer program to estimate gene genealogies. Molecular Ecology. 9(10): 1657-1660

Clement, M., Snell, Q., Walker, P., Posada, D., and Crandall, K. (2002). TCS: Estimating gene genealogies. Parallel and Distributed Processing Symposium, International Proceedings, 2, 184.

Corbet, P.S. (1962). “A biology of dragonflies”. Witherby, London. 274 pp.

Darriba, D., Taboada GL, Doallo R and Posada D. (2012). "jModelTest 2: more models, new heuristics and parallel computing". Nature Methods. 9(8):772.

Excoffier, L. and Lischer, H.E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 10: 564-567.

Feng, H.Q., Wu, K.M., Ni, Y.X., Cheng, D.F., Guo, Y.Y. (2006). Nocturnal migration of dragonflies over the Bohai Sea in northern China. Ecological Entomology. 31(5):511-520.

Fraser, F.C. (1936). Odonata. Vol. III. The Fauna of British India including Ceylon and Burma. Taylor and Francis, London. 461 pp.

Garrison, R.W., Ellenrieder, N., Louton, J.A. (2006). Dragonfly genera of the New World.

Hopkins University Press, Baltimore.

Gibo, D.L. (1981). Some observations on slope soaring in *Pantala flavescens* (Odonata: Libellulidae). Journal of the New York Entomological Society. 89(3):184-187.

Hebert, P.D.N., Ratnasingham, S., DeWaard, J.R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society B: Biological Sciences. 270:S96-S99

Huelsenbeck, J. P. and Ronquist., F. (2001). MRBAYES: Bayesian inference of phylogeny. Bioinformatics. 17:754-755.

Hobson, K.A., Anderson, R.C., Soto, D.X., Wassenaar, L.I. (2012). Isotopic evidence that dragonflies (*Pantala flavescens*) migrating through the Maldives come from the northern Indian subcontinent. PLoS One. 7(12):e52594.

Ichikawa, Y., Watanabe, M. (2014). Changes in the number of eggs loaded in pantala flavescens females with age from mass flights (Odonata: Libellulidae). Zoological Science. 31(11):721-724.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12), 1647-1649.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G. (2007). Clustal W and Clustal X version 2.0. Bioinformatics. 23:2947-2948.

Librado, P. and Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25:1451-1452.

Maddison, W.P. and D.R. Maddison (2015). Mesquite: a modular system for evolutionary analysis. Version 3.02 http://mesquiteproject.org

May, M.L. (2013). A critical overview of progress in studies of migration of dragonflies (Odonata: Anisoptera), with emphasis on North America. Journal of Insect Conservation. 17(1):1-15.

Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1 – 8.

Peakall, R. and Smouse, P.E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6, 288-295.

Peakall, R. and Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 28, 2537-2539.

Pilgrim, E.M. and von Dohlen, C.D., (2008). Phylogeny of the Sympetrinae (Odonata: Libellulidae): further evidence of the homoplasious nature of wing venation. Systematic Entomology. 33:159-174.

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J. (2014). Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer

Ronquist, F. and Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19:1572-1574.

Russell, R.W., May, M.L., Soltesz, K.L., Fitzpatrick, J.W. (1998). Massive swarm migrations of dragonflies (Odonata) in eastern North America. American Midland Naturalist. 140(2):325-342.

Russell, R.W., Wilson, J.W. (1997). Radar-observed “fine lines” in the optically clear boundary layer: Reflectivity contributions from aerial plankton and its predators. Boundary-Layer Meteorology. 82(2):235-262.

Samways, M.J., Osborn, R. (1998). Divergence in a transoceanic circumptropical dragonfly on a remote island. Journal of Biogeography. 25:935-946.

Schorr, M., Paulson, D. (2015). World Odonata List. http://www.pugetsound.edu.

Suhling, F., Schenk, K., Padeffke, T., Martens, A. (2004). A field study of larval development in a dragonfly assemblage in African desert ponds (Odonata). Hydrobiologia. 528(1-3):75-85.

Sukumaran, J. and Holder, M.T. (2010). DendroPy: A Python library for phylogenetic computing. Bioinformatics. 26: 1569-1571.

Ware, J.L., May, M.L., Kjer, K.M. (2007). Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): an exploration of the most speciose superfamily of dragonflies. Molecular Phylogenetics and Evolution. 45(1): 289–310.

Ware, J. L., Ho, S. Y. W., Kjer, K. M. (2008). Divergence dates of libelluloid dragonflies (Odonata: Anisoptera) estimated from rRNA using paired-site substitution models. Molecular Phylogenetics and Evolution. 47:426-432.

Whitlock, M.C., and Mccauley, D.E. (1999). Indirect measures of gene flow and migration: F(ST) ≠ 1/(4Nm + 1). Heredity. 82(2):117-125.

Wikelski, M., Moskowitz, D., Adelman, J.S., Cochran, J., Wilcove, D.S., May, M.L. (2006). Simple rules guide dragonfly migration. Biology Letters. 2(3):325-329.

Zhan, S., Merlin, C., Boore, J.L., Reppert, S.M. (2011). The monarch butterfly genome yields insights into long-distance migration. Cell. 147(5):1171-1185.

Zwickl, D.J. (2006). Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation, The University of Texas at Austin.

Table 1:

COI Primers	Sequence	Tm ºC
LCO (forward)	5' GGTCAACAAATCATAAAGATATTGG 3'	58.0º
HCO (reverse)	5' TAAACTTCAGGGTGACCAAAAAATCA 3'	59.9º
coi1709 (forward)	5' TAATTGGAGGATTTGGAAATTG 3'	55.2º
coi2191 (reverse)	5' CCYGGTARAATTARAATRTARACTTC 3'	59.1º

Figure 1: Mr Bayes Bayesian majority rule consensus tree (Based on CO1). Known locations of samples are indicated in parentheses. (G# = GenBank Sequence, B# = BOLD Systems Sequence)

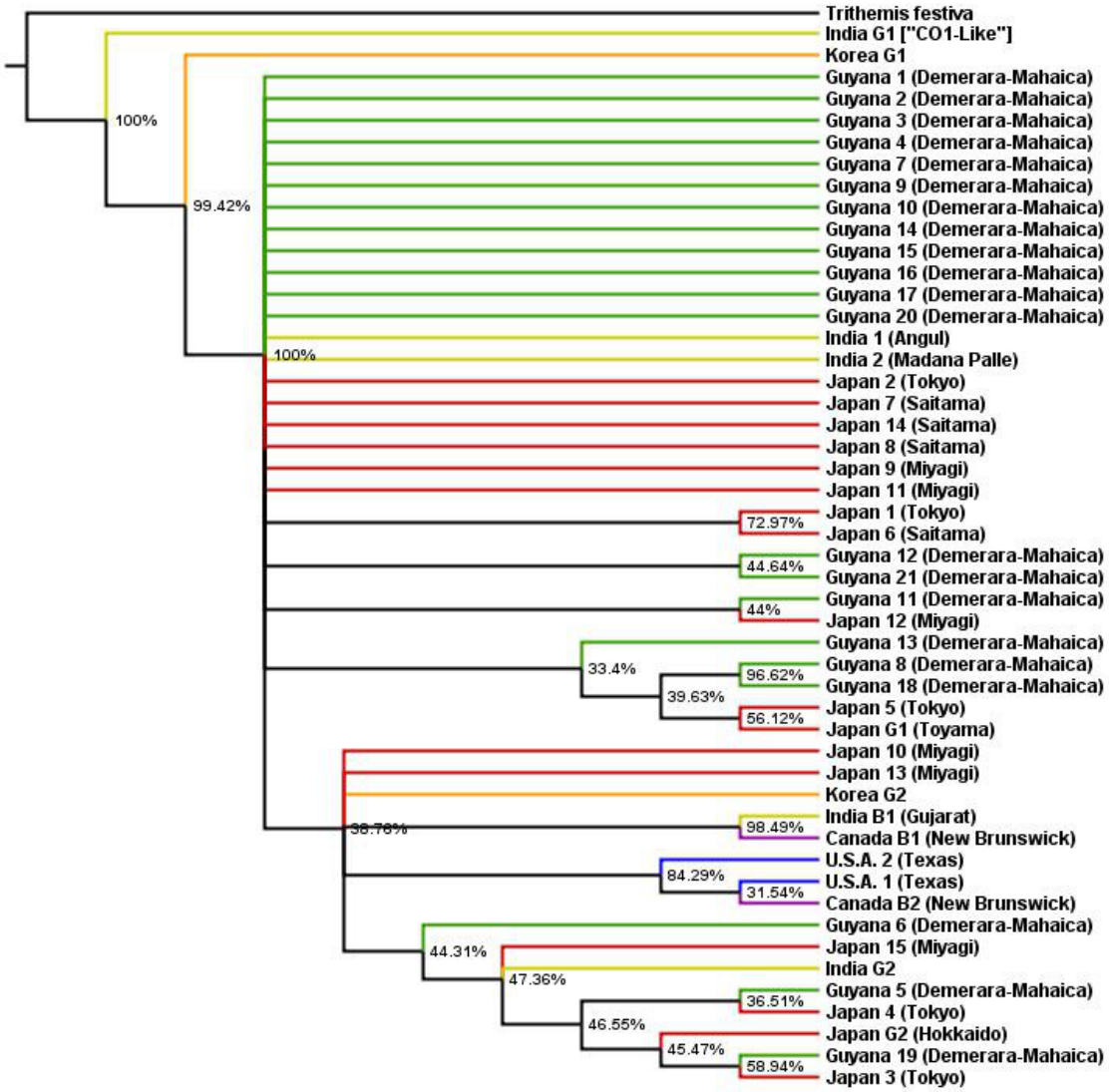

Figure 2: GARLI majority rule consensus tree (Based on CO1). Known locations of samples are indicated in parentheses. (G# = GenBank Sequence, B# = BOLD Systems Sequence)

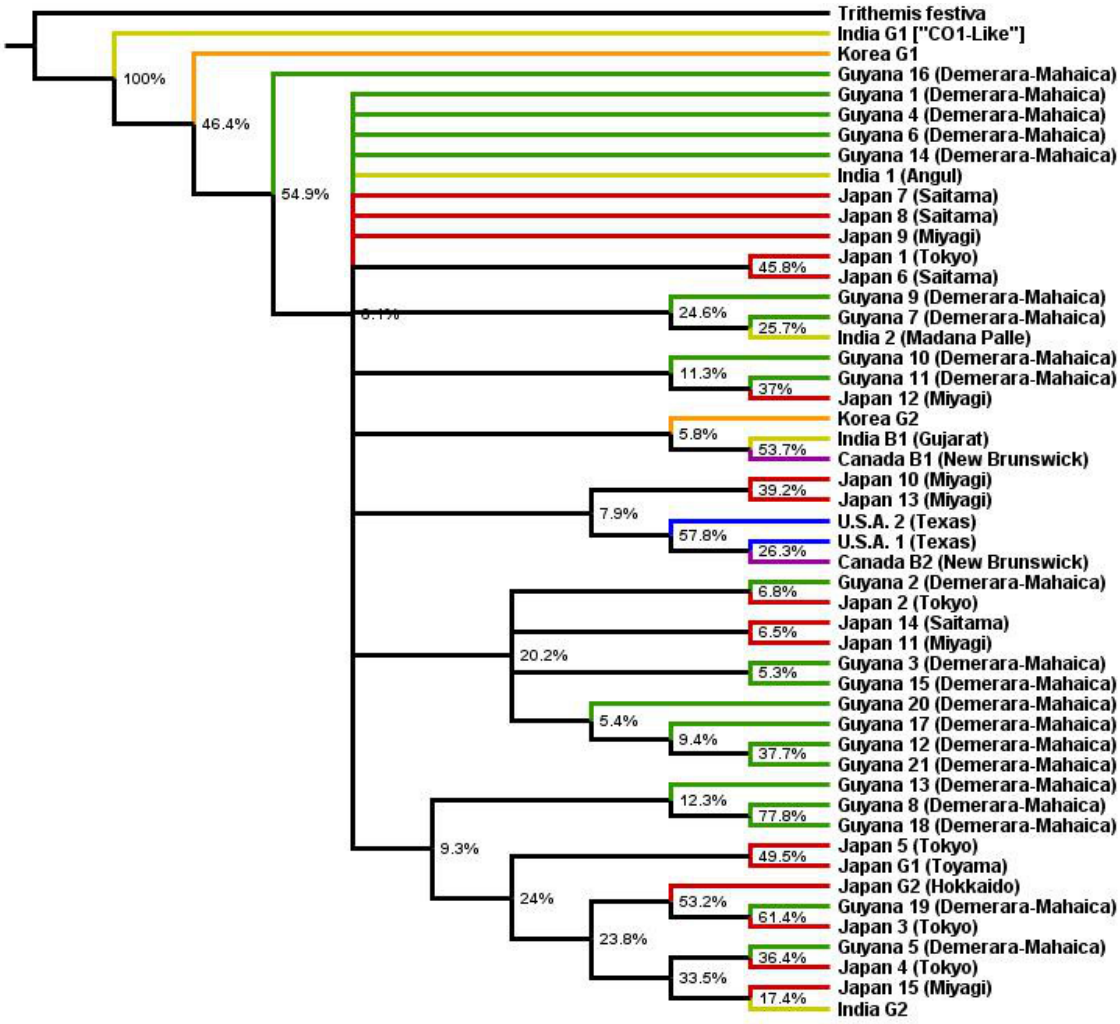

Figure 3: PopART Minimum Spanning Haplotype Network. All haplotypes based on COI gene except for India G1 which is "COI-like". Numbers in parentheses indicated the number of changes between haplotypes.

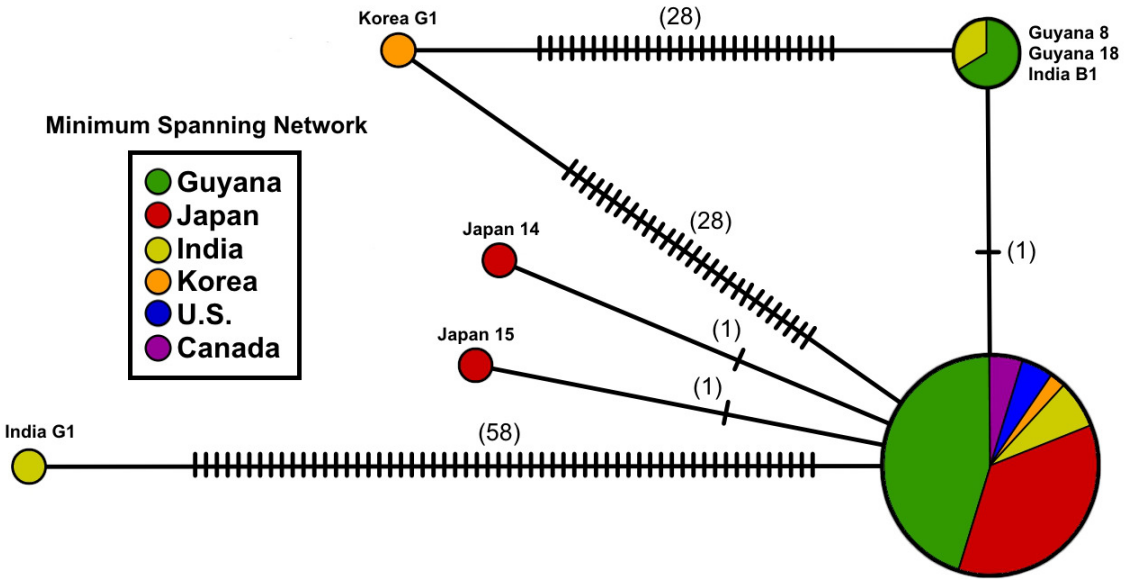

Figure 4: PopART TCS Haplotype Network. All haplotypes are based on COI gene except for India G1 which is "COI-like". Numbers in parentheses indicated the number of changes between haplotypes.

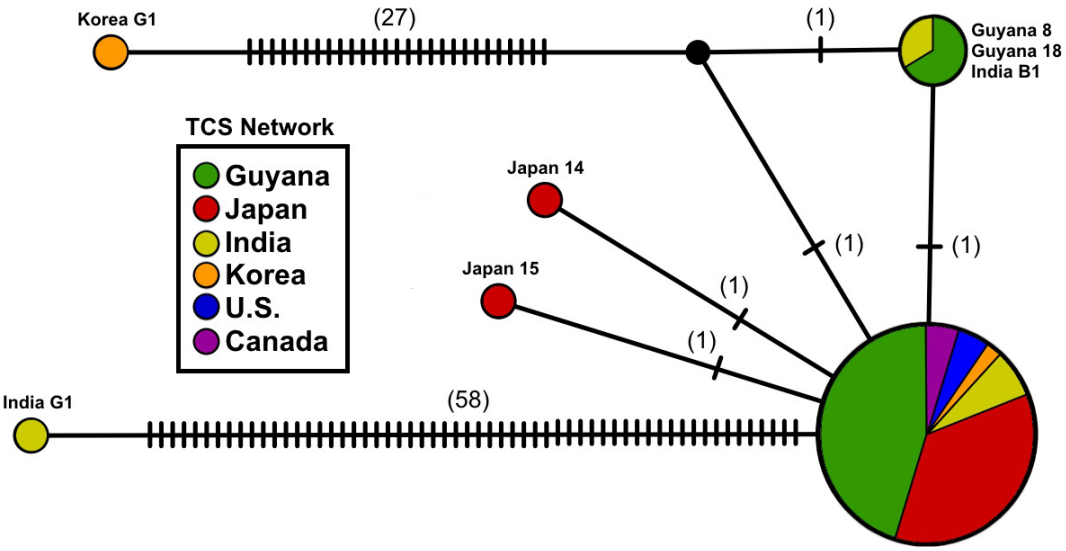

Figure 5: TCS 1.21 Haplotype Network. All haplotypes are based on COI gene except for India G1 which is “COI-like”.

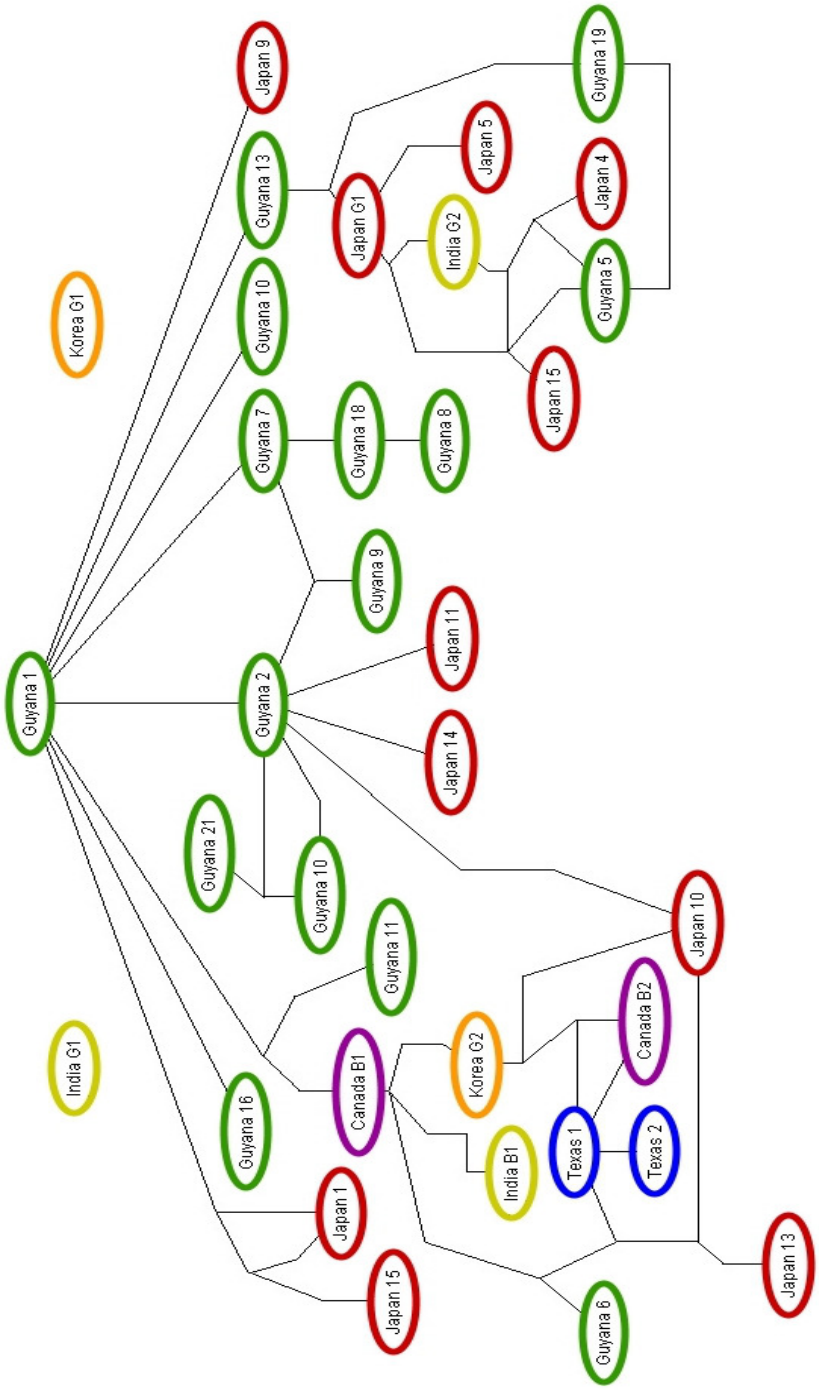

Figure 6: Distribution of individuals used in this study. The dotted line represents the suggested India to Africa migration route.