Historical relationships of three enigmatic phasianid genera (Aves: Galliformes) inferred using phylogenomic and mitogenomic data

Ning Wang^{a,b,*}, Peter A. Hosner^{a,c}, Bin Liang^{a,b}, Edward L. Braun^a, Rebecca T. Kimball^{a,*}

^a *Department of Biology, University of Florida, Gainesville, FL 32611, USA*

^b *Ministry of Education Key Laboratory for Tropical Plant and Animal Ecology, College of Life Sciences, Hainan Normal University, Haikou 571158, China.*

^c *Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA*

* Corresponding authors:

Rebecca T. Kimball, Department of Biology, University of Florida, Gainesville, FL 32611 USA. E-mail: rkimball@ufl.edu.

Ning Wang, Department of Biology, University of Florida, Gainesville, FL 32611, USA. E-mail: ningwang83@hotmail.com.

ABSTRACT

The phylogeny of the Phasianidae (pheasants, partridges, and allies) has been studied extensively. However, these studies have largely ignored three enigmatic genera because of scarce DNA source material and limited overlapping phylogenetic data: blood pheasants (*Ithaginis*), snow partridges (*Lerwa*), and long-billed partridges (*Rhizothera*). Thus, phylogenetic positions of these three genera remain uncertain in what is otherwise a well-resolved phylogeny. Previous studies using different data types place *Lerwa* and *Ithaginis* in similar positions, but the absence of overlapping data means the relationship between them could not be inferred.

Rhizothera was originally described in the genus *Perdix* (true partridges), although a partial cytochrome *b* (CYB) sequence suggests it is sister to *Pucrasia* (koklass pheasant). To identify robust relationships among *Ithaginis*, *Lerwa*, *Rhizothera*, and their phasianid relatives, we used 3692 ultra-conserved element (UCE) loci and complete mitogenomes from 19 species including previously hypothesized relatives of the three focal genera and representatives from all major phasianid clades. We used DNA extracted from historical specimen toepads for species that lacked fresh tissue in museum collections. Maximum likelihood and multispecies coalescent UCE analyses strongly supported *Lerwa* sister to a large clade which included *Ithaginis* at its base, and also including turkey, grouse, typical pheasants, tragopans, *Pucrasia*, and *Perdix*. *Rhizothera* was also in this clade, sister to a diverse group comprising *Perdix*, typical pheasants, *Pucrasia*, turkey and grouse. Mitogenomic genealogies differed from UCEs topologies, supporting a sister relationship between *Ithaginis* and *Lerwa* rather than a grade. The position of *Rhizothera* using mitogenomes depended on analytical choices. Unpartitioned and codon-based analyses placed *Rhizothera* sister to a tragopan clade, whereas a partitioned DNA model of the mitogenome was congruent with UCE results. In all mitogenome analyses, *Pucrasia* was sister to a clade including *Perdix* and the typical pheasants with high support, in contrast to UCEs and published nuclear intron data. Due to the strong support and consistent topology provided by all UCE analyses, we have identified phylogenetic relationships of these three enigmatic, poorly-studied, phasianid taxa.

Keywords: ultraconserved elements, mitochondrial genome, *Ithaginis*, *Lerwa*, *Rhizothera*.

1. Introduction

The well-known avian order Galliformes (chickens, turkey, quail, and allies) comprises 299 extant species (Gill and Donsker, 2016) that exhibit extraordinary diversity in morphology, ecology, and behavior. Previous large-scale studies on galliform phylogeny recovered identical family-level affinities [i.e., (Megapodiidae, (Cracidae, (Numididae, (Odontophoridae, Phasianidae)))] with high bootstrap support (e.g., Crowe et al., 2006; Hosner et al., 2016a; Kimball and Braun, 2014; Wang et al., 2013). The most species-rich family, Phasianidae (with 183 species; Gill and Donsker, 2016) underwent numerous rapid radiations, and has been the focus of the majority of phylogenetic studies (e.g., Hosner et al., 2016a; Kimball et al., 2011; Kimball and Braun, 2014; Shen et al., 2014; Wang et al., 2013). Although substantial progress has been made in resolving phylogenetic history of the Phasianidae, with next-generation sequencing approaches helping to resolve conflicts among previous studies (e.g., Hosner et al., 2016a; Persons et al., 2016; Sun et al., 2014; but see Meiklejohn et al. 2016), the relationships among several phasianid genera; e.g., the monotypic *Ithaginis cruentus* (blood pheasant), *Lerwa lerwa* (snow partridge) and *Rhizothera longirostris* (long-billed partridge; this is sometimes considered two species; Gill and Donsker, 2016), are still not clear. These enigmatic taxa have been sampled for little data and/or they have not been analyzed with an appropriate set of taxa to fully resolve their phylogenetic position.

Ithaginis cruentus is widely distributed in the southern Himalayas, the eastern edge of the Tibetan plateau, and the Qinling Mountains of China (Yang et al., 1994; Zhan et al., 2011), where it inhabits high-altitude coniferous or mixed forests and scrub up to 4600 m (Johnsgard, 1999). This sexually dimorphic species is also highly polymorphic, particularly considering male

plumage, with nine to fourteen subspecies recognized by various authorities (Cheng, 1978; Delacour, 1951; Howard and Moore, 1980; Johnsgard, 1999; Madge and McGowan, 2002; Yang et al., 1994). Zhan et al. (2011) sampled *Ithaginis* within China (including 10 subspecies) and identified four major populations, including support for two distinct plumage groups (the red-winged and green-winged blood pheasants; Cheng, 1978; Johnsgard, 1999), suggesting deep divergences within this species.

Traditionally, *Ithaginis* is included in the “Tragopan and allies” group (Johnsgard, 1986) that also includes *Tragopan spp.* (horned pheasants), *Lophophorus spp.* (monal pheasants), and *Pucrasia macrolopha* (koklass pheasant), although many recent studies have found that these taxa do not form a monophyletic group (reviewed by Wang et al., 2013). Due to the shared small bill and lanceolate-shaped feathers, *Pucrasia macrolopha* has been suggested to be its closest relative (Madge and McGowan, 2002). Morphological analyses (Dyke et al., 2003) suggest an alternative placement of the blood pheasant at the base of the major “partridges” and the Odontophoridae, although this result differs from all molecular studies which clearly separate the Odontophoridae from various partridge taxa.

Studies using molecular data have resulted in several different phylogenetic hypotheses for *Ithaginis*. Crowe et al. (2006) identified it as sister to Gallopheasants (the typical pheasants). Complete mitochondrial sequences (Meiklejohn et al., 2014; Shen et al., 2014) suggest *Ithaginis* is instead basal to the "erectile" clade (a clade including typical pheasants, turkeys, *Pucrasia* and allies; Kimball and Braun, 2008). The basal position of *Ithaginis* to the erectile clade is also strongly supported by Wang et al. (2013), which included two mitochondrial regions as well as six nuclear introns. However, a conflicting position occurs with the nuclear data of Shen et al. (2014) that strongly supports *Ithaginis* as sister to *Pucrasia* (a position also suggested by Madge

and McGowan 2002). The seven nuclear loci used by Shen et al. (2014) included five protein coding genes, and differences between intron and coding data have been observed in birds (e.g., Jarvis et al., 2014) which may explain the conflict.

Like *Ithaginis*, *Lerwa* also exhibits a high altitude distribution in the Himalayas (from 3000m to 5500m, Cheng et al., 1978). As a large-bodied and heavily barred alpine partridge, it has been suggested that this monomorphic species might be an aberrant francolin (Madge and McGowan, 2002). However, its downy chick is remarkably similar to *Ithaginis* (Madge and McGowan, 2002; Potapov, 2000), whereas its whistling calls and eggs suggest a relationship to another large-bodied, Asian alpine genus, *Tetraogallus* (snowcocks). However, these attributes may be merely convergent and indicative of a similar lifestyle (Johnsgard, 1988). In the absence of DNA sequence data, Stein et al. (2015) considered *Lerwa* related to *Meleagris* (turkey), in part due to their shared species of chewing lice (*Ischnocera*) (Mey, 2006) and the same number of tarsal spurs on adult males (Davison, 1985), which is consistent with the suggestion of Potapov (2000) that *Lerwa* has no clear systematic affinities within Asia. Only one molecular phylogeny has included *Lerwa*, which places *Lerwa* at the base of the erectile clade using 4,817 UCE (ultra-conserved element) loci (Hosner et al., 2016a). However, that study lacked *Ithaginis*, and because no previous study has included both *Ithaginis* and *Lerwa*, the relationship between them has not been inferred with molecular data (assuming *Ithaginis* is at or near the base of the erectile clade, as it is in Meiklejohn et al., 2014 and Wang et al., 2013).

Rhizothera longirostris, is an elusive, large and sexually dimorphic partridge of tropical forest in the Malayan Peninsula and Greater Sundas. It is typically separated into either two subspecies (e.g., Clements et al., 2015) or else two species (e.g., Gill and Donsker, 2016): the widespread *R. longirostris*, and *R. dulitensis* that is restricted to mountains of central Borneo.

The original description of *Rhizothera* (Temminck 1815, cited in Davison 1999) placed it in the genus *Perdix* (true partridges). Johnsgard (1988) suggested that it might have evolved from an early *Arborophila*-like stock (hill partridges), while Crowe and Crowe (1985) suggest possible affinities with *Francolinus* (francolins). With limited molecular data [~700bp of mitochondrial cytochrome *b* (CYB) sequence], some recent supermatrix analyses have placed *Rhizothera* sister to *Pucrasia macrolopha* (Burleigh et al., 2015; Jetz et al., 2012; Stein et al., 2015), though the position of the *Rhizothera*-*Pucrasia* clade varies among these studies.

More generally, the position of *Pucrasia* has varied among previous studies, regardless of the relationship between *Pucrasia* and *Rhizothera*. Several studies using nuclear intron or UCE data have supported that *Pucrasia* is sister to *Meleagris* and the Tetraoninae (grouse) (e.g., Hosner et al., 2016a; Kimball and Braun, 2014; Wang et al., 2013). However, using complete mitogenomes, Meiklejohn et al. (2014) placed *Pucrasia* sister to a *Perdix*-Gallopheasant clade; the mitochondrial data of Wang et al. (2013) also support this position. Given that the phylogenetic position of *Pucrasia* appears to differ between mitochondrial and nuclear data, it is unclear if the putative relationship between *Pucrasia* and *Rhizothera* is also specific to mitochondrial data (since the only data currently available for *Rhizothera* are mitochondrial), or if it will hold true in nuclear datasets as well.

Having well-resolved phylogenies, that are sampled for major groups, can be important in analyses aimed at understanding trait evolution and biogeographic patterns (e.g., Wang et al., 2016), making it important to place unresolved taxa. Therefore, in order to test alternative hypotheses about the position of *Ithaginis*, *Lerwa* and *Rhizothera*, we used 3692 ultra-conserved element (UCE) loci (about 1.59 million bp aligned) and mitogenomes from 19 representative galliform species. We selected these taxa to represent hypothesized *Ithaginis*, *Lerwa* and

Rhizothera sister taxa, as well as all major phasianid clades and outgroups. UCEs are widely distributed throughout genomes, exhibit limited saturation, and are easy to align (McCormack et al., 2012; Meiklejohn et al., 2016). Although UCEs are highly conserved across distantly related taxa, their flanking regions contain abundant variations that are capable of resolving shallow-level relationships (Smith et al., 2014) and over short internodes (Meiklejohn et al., 2016). Thus they are suitable for a wide variety of questions at varying evolutionary depths (e.g., McCormack et al., 2013; Smith et al., 2014; Sun et al., 2014).

Mitochondrial regions often yield conflicting topologies when compared to nuclear data. However, because several previous studies have relied heavily on mitochondrial markers, we also included complete or near-complete mitogenomes for each species. Whole mitogenomes often yield much stronger phylogenetic signal than one or a few mitochondrial genes (e.g., Meiklejohn et al., 2014), which may help determine if differing mitochondrial topologies are due to lack of phylogenetic signal or cyto-nuclear discordance. In some cases, we obtained a mitogenome for a species that already had a published mitogenome. Since mitochondrial data are often used to identify deep divergences that may represent distinct species (e.g., Rach et al., 2008), we also included these for some analyses to assess whether there may be deep divergences that may warrant further study.

We address the following questions: 1), what is the phylogenetic position of *Ithaginis*, *Lerwa* and *Rhizothera* within the phasianidae; 2), does increased taxon sampling (inclusion of *Rhizothera*) remove the conflict between nuclear and mitochondrial data in the placement of *Pucrasia* (compare Meiklejohn et al., 2014 to Hosner et al., 2016a; Kimball and Braun, 2014; Wang et al., 2013); and 3), for cases when two intraspecific mitogenomes are present, what were the levels of mitogenomic divergence among different individuals within species and are

possible cryptic species present?

2. Methods

2.1 Collection of sequence data

2.1.1 Ultraconserved element data

Previous studies suggest that *Rhizothera* is within the erectile clade, and that *Lerwa* and *Ithaginis* are at the base of the erectile clade or within the erectile clade (e.g., Hosner et al., 2016a; Shen et al., 2014; Wang et al., 2013), thus we focused taxon sampling within that group. In addition to the focal taxa *Ithaginis*, *Lerwa*, and *Rhizothera* (for which we included two samples collected from Myanmar and Borneo), we selected nine species representing the major lineages of the erectile clade. We also included six species from major phasianid lineages outside the erectile clade, and one odontophorid as an outgroup (Supplementary Material Table A.1). The resulting dataset included 20 taxa from 19 species.

DNA was extracted from toepads of museum specimens for *Ithaginis* and *Rhizothera* using the Gentra Puregene® DNA purification kit (Qiagen Inc., Valencia CA), following standard manufacturers protocols. The UCE data were prepared by RAPiD Genomics (Gainesville, FL) using a modified approach described by Faircloth et al. (2012). Illumina TruSeq libraries were prepared following the manufacturer's protocol (Illumina Inc., San Diego, CA, USA) as modified by Faircloth and Glenn (2012). Libraries were enriched for 5060 UCE loci targeted by 5472 probes (Mycroarray, Ann Arbor, MI;

<http://www.mycroarray.com/mybaits/mybaits-UCes.html>) and PE100 reads were generated using an Illumina (San Diego, CA) HiSeq 2500 lane. We applied Trimmomatic (Bolger et al., 2014) for reads quality control and used Trinity r20131110 (Grabherr et al., 2011) to assemble the data. Newly collected data was combined with data from *Gallus gallus* (chicken) that was extracted from published genomes (Hillier et al., 2004), and with data from Sun et al. (2014), Hosner et al. (2016a) and Persons et al. (2016). We extracted UCE loci from resulting contigs with the PHYLUCE 1.5 pipeline (Faircloth et al., 2012) and aligned them using MAFFT 7 (Katoh et al., 2002; Katoh and Standley, 2013). Raw sequence reads are archived at GenBank under BioProject PRJNA358478.

Since large amounts of missing data can lead to erroneous phylogenetic estimation in multispecies coalescent frameworks (e.g., Hosner et al., 2016a; Roure et al., 2013), only UCE loci sampled for >75% of focal taxa were retained for phylogenetic analysis (Hosner et al., 2016a). These alignments were examined by eye in Geneious 6.1.2 (Biomatters Ltd., 2013) and we removed likely assembly or alignment errors at the 5' or 3' ends prior to subsequent analyses (Meiklejohn et al., 2016). The resulting matrix included 3692 UCE loci with an average length of ~432bp and <25% missing data.

2.1.2 Mitogenome data

Although the libraries used for UCE sequencing were enriched for UCEs, in some cases substantial numbers of mitochondrial sequences were also obtained (particularly when DNA was extracted from mitochondrially-enriched tissues). Using sequence data collected for this study (or in our previously published studies; Sun et al., 2014, Hosner et al., 2016a and Persons et al.,

2016), we used read-mapping in Geneious 6.1.2 (Biomatters Ltd., 2013) and the complete chicken mitochondria (Desjardins and Morais, 1990) to assemble mitogenomes that generated 11 new mitogenomic sequences (Supplementary Material Table A.1). Sequences are available at GenBank XXXXXX-XXXXXX.

We combined this with published mitochondrial sequences to either provide a second mitochondrion for some species (six species were represented by two individuals), or for species in which our sequencing efforts resulted in too few mitochondrial reads to assemble a high quality mitochondrial genome (Supplementary Material Table A.1). We used Mesquite 3.04 (Maddison and Maddison, 2015) to check individual protein-coding gene alignments for insertion–deletion events and premature stop codons to ensure the absence of numt sequences. We excluded stop codons and the programmed frameshift in ND3 (Mindell et al., 1998; Russell and Beckenbach, 2008) to generate a final data matrix containing two rRNAs and 13 protein-coding genes with 14086 bp (20 taxon dataset) or 14088 bp (25 taxon dataset).

2.2. Phylogenomic analyses

2.2.1. Ultraconserved element data

We conducted unpartitioned and partitioned ML analyses of the concatenated UCE alignment in RAxML 8.2.3 (Stamatakis, 2014) with the GTRGAMMA model and assessed support using 500 ML bootstrap replicates. The partitioning scheme was generated using the rclusterf searching algorithm and AICc criterion for model selection in PartitionFinder 2.0.0-pre11 (Lanfear et al., 2012). We also estimated a multispecies coalescent tree with the UCE data.

Due to the problems of gene trees estimated from sequences with few informative sites (Hosner et al., 2016a; Meiklejohn et al., 2016), for this analysis we used the 25% most informative loci (Hosner et al., 2016a) resulting in 961 loci (each locus had at least 15 parsimony informative sites). For each locus, we produced one optimal tree and 500 ML bootstrap trees using Garli 2.0 (Zwickl, 2006), implementing the best-fit model for each locus based on the AICc (MrAIC, Nylander, 2004). Branches with near zero lengths were treated as polytomies in Garli to accommodate the influence of random resolution of such branch lengths on the construction of species tree (Meiklejohn et al., 2016). The bootstrap gene trees as well as the optimal trees were then used as input for reconciliation with ASTRAL 4.10.2 (Mirarab et al., 2014; Mirarab and Warnow, 2015; Sayyari and Mirarab, 2016), which performs well when using the most variable UCEs (Hosner et al., 2016a; Meiklejohn et al., 2016).

2.2.2. Mitochondrial genome data

We conducted unpartitioned and partitioned ML analyses in RAxML 8.0.2 (Stamatakis, 2014) under the GTRGAMMA model using a complete data matrix (25 taxa, with duplicate mitogenomes for some species) and a reduced data matrix (20 taxa, with one representative to match each of the taxa sampled for UCEs). The partitioning scheme was generated using the greedy search algorithm and AICc criterion for model selection in PartitionFinder 2.0.0-pre11 (Lanfear et al., 2012). 500 replicate bootstraps were used to assess clade support. Since the use of better-fitting models for mitochondrial data may facilitate the phylogenetic estimation (e.g., Braun and Kimball, 2002; Powell et al., 2013), unpartitioned and partitioned ML analyses [the latter with both a DNA model (assigning each partition with the optimal DNA substitution model

provided by PartitionFinder) and a DNA+Codon model (DNA model for rRNAs and codon model for the combined 13 protein coding genes, hereafter mixed model)] were also estimated in IQtree 1.4.1 (Chernomor et al., 2016; Minh et al., 2013; Nguyen et al., 2015) for both datasets. We assessed the divergence within species using uncorrected p -distances calculated in PAUP*4.0b10 (Swofford, 2003).

3. Results

3.1. Relationships of enigmatic taxa

Our concatenated analyses of the UCE data resulted in a completely resolved phylogenetic tree (Fig. 1). Both unpartitioned and partitioned maximum likelihood analyses recovered identical topologies, with all clades obtaining 100% bootstrap support. The species tree from ASTRAL also recovered the same topology as the ML tree, although bootstrap support was lower for some nodes (Fig. 1). In particular, *Lerwa* and *Ithaginis* formed a grade at the base of the erectile clade; *Rhizothera* was sister to a large group that includes *Pucrasia-Meleagris*-Grouse and *Perdix*-Gallopheasants. Additionally, the non-erectile clade (i.e., chickens and allies, Wang et al., 2013) was recovered with 100% support; the position of *Haematortyx* as sister to *Pavo-Argusianus* was consistent with other species tree analyses of this clade (Hosner et al., 2016a; Sun et al., 2014).

3.2. Mitochondrial-Nuclear conflicts

Phylogenetic trees estimated using the mitogenome data differed from the UCE trees in several aspects (Figs. 1 and 2). First, all mitogenomic ML analyses supported a sister relationship between *Ithaginis* and *Lerwa* (Figs. 2 and 3); this *Ithaginis-Lerwa* sister clade was basal to the erectile clade. This was in contrast to the UCE tree where *Ithaginis* and *Lerwa* formed a grade at the base of the erectile clade (Fig. 1). Second, *Pucrasia* was sister to the *Perdix*-Gallupheasants clade with high support in the mitogenomic trees, in conflict with the UCE tree (Fig. 1). Third, placement of *Rhizothera* was sensitive to mitogenomic partitioning strategy. In analyses with un-partitioned DNA and using both partitioned DNA and mixed models in IQtree, *Rhizothera* was sister to the *Tetraophasis-Tragopan* clade (Fig. 2A). However, in partitioned RAxML analyses, *Rhizothera* was sister to a larger clade including *Meleagris*, *Perdix*, *Pucrasia* and others (Fig. 2B), consistent with the UCE data, albeit with lower support (Fig. 1). Finally, there were several differences outside of the erectile clade. For example, the unpartitioned mitogenomic ML tree in RAxML (20-taxa dataset) and IQtree (both 20 and 25-taxa dataset) marginally supported a non-erectile clade (Fig. 2A and Supplementary Material tree file), consistent with the UCE data (and many other analyses of nuclear data; Bonilla et al., 2010; Hosner et al., 2016a; Kimball and Braun, 2014; Wang et al., 2013), although species relationships within the non-erectile clade differed from that of the UCE data. However, all other mitogenomic analyses did not recover the non-erectile clade, instead forming a grade (Figs. 2B and 3, Supplementary Material tree file). Regardless, the differences outside the erectile clade were poorly supported in the mitogenomic analyses and were often sensitive to specific analysis (e.g., Figs. 2 and 3, Supplementary Material tree file), suggesting that little confidence should be placed on these mitochondrial relationships.

3.3. Levels of mitogenome divergence within different species

Using five additional mitogenome sequences, the divergence (uncorrected *p* distances) between individuals within species ranged from 0.00086 (*Perdix*) to 0.03024 (*Ithaginis*) (Table 1). The divergence was 0.01549 for the two individuals in *Rhizothera*, which is lower than most interspecific distances in birds (Ward, 2009). Thus, it is likely that the individuals of *Rhizothera* from Myanmar and Borneo sampled for our study might not be sufficiently divergent given that they are ascribed to the same subspecies and do not differ in phenotype. However, the samples of *Ithaginis*, which is highly polytypic with 12 named subspecies (Gill and Donsker, 2016), exhibited a large mitogenome divergence. Our *Ithaginis* sample was collected in Nepal, though the published mitogenome (i.e., NC_018033, Zeng et al., 2013) does not specify collecting locale. However, the published mitogenome is identical to published control region and cytochrome b sequences of an individual from Yunnan province (Zhan et al., 2011), and thus this sample likely represents a different morphological and genetic group from our sample. These results suggest a more detailed exploration of whether at least some subspecies within *Ithaginis* should be treated as full species is warranted. Otherwise, other comparisons within species exhibited relatively low mitogenomic divergence, as was expected.

4. Discussion

4.1. Phylogenomics place three enigmatic phasianid genera

In the last decade, molecular phylogenetics, especially the analysis of multilocus datasets,

have greatly improved understanding of phasianid relationships (reviewed in Wang et al., 2013). However, the phylogenetic positions of certain species have remained unclear. One potential reason for the limited information about relationships of certain taxa is due to the unavailability of fresh tissues from these species. A solution to this problem is the use of specimens from natural history collections in museums, which have been collected over a much longer period of time and cover much greater taxonomic diversity than tissue collections. However, it has been difficult to realize the promise of museums as storehouses of genetic material (Houde and Braun, 1988; Payne and Sorenson, 2002; Wandeler et al., 2007) given the degradation of DNA in most historical samples. Historical samples are valuable and often unique, therefore destructive sampling for genetic analyses may be difficult to justify if DNA degradation makes the likelihood of successful data collection low (Graves and Braun, 1992). However, sequence capture methods now allow for the reliable collection of thousands of loci from older specimens in natural history collections (Hosner et al., 2016a; McCormack et al., 2015; Sun et al., 2014), so it is now much more justifiable to conduct limited destructive sampling of these specimens. Here we were able to place several enigmatic phasianids using thousands of nuclear loci and complete mitogenomes generated by sequence capture with DNA extracted from toepads of museum specimens.

Our analyses of UCE data recovered similar relationship among phasianids as previous studies using multi-locus datasets. In general, all analyses (concatenated gene tree and species tree) based on UCE data recovered the same topology, and found the erectile and non-erectile clades with 100% support. However, within those clades the species tree showed low support for some relationships (Fig. 1). Although multispecies coalescent methods represent a valuable paradigm in phylogenetics (Edwards et al., 2016), it is also known that coalescent species trees

based on gene tree summation may not always perform significantly better than concatenation methods (Patel et al., 2013; Tonini et al., 2015), and lower support for many relationships in species trees is typical (e.g., Lambert et al., 2015).

In contrast with the analyses of nuclear data by Shen et al. (2014), our UCE data instead agree with the nuclear intron data of Wang et al. (2013) in placing *Ithaginis* at the base of the erectile clade, as also occurs when using complete mitochondrial genome sequences (Figs. 2 and 3; Bao et al., 2010; Meiklejohn et al., 2014; Meng et al., 2008; Shen et al., 2014). Furthermore, we resolved the relative positions of *Ithaginis* and another poorly known taxa, *Lerwa*, which form a grade at the base of the erectile clade. These are both heavy bodied, alpine species, though *Ithaginis* is strongly sexually dimorphic, while *Lerwa* is monomorphic. The suggestion that *Lerwa* is not clearly related to other Asian taxa (Potapov, 2000) might be interpreted as excluding a sister relationship with *Ithaginis*.

Finally, in contrast with studies analyzing a small region of mitochondrial data (e.g., Burleigh et al., 2015; Jetz et al., 2012; Stein et al., 2015), our results confidently place *Rhizothera* at the base of a larger clade that includes *Pucrasia* as well as a number of other taxa, although this position was not universally supported in mitogenome analyses.

4.2. Mitochondrial-Nuclear conflicts

Cyto-nuclear discordance is common, though determining its causes can be difficult (Toews and Brelsford, 2012). Among the differences between the topologies of the UCE and mitogenome data, many were outside the erectile clade. Whether the non-erectile group forms a clade or a grade has been difficult to establish, as have relationships within the non-erectile clade

(e.g., Crowe et al., 2006, Kimball and Braun, 2014; Wang et al., 2013). This appears to be due to rapid radiation and short internodes among these problematic groups and the weak power to resolve their relationships based on smaller data sets. This may be why the mitogenomes, which have fewer variable sites and higher homoplasy than UCEs (Meiklejohn et al., 2016), exhibited low bootstrap support and differences when compared with the UCEs outside of the erectile clade. Given the lower support for these relationships using the mitogenome (as well as the more limited taxon sampling outside of the erectile clade), congruence with the UCEs cannot be rejected for the non-erectile clade.

However, there were two relationships in the erectile clade that did exhibit well-supported and consistent conflict between the UCEs and the mitogenome - the placement of *Pucrasia* and the relationship between *Ithaginis* and *Lerwa*. While no previous molecular study has included both *Ithaginis* and *Lerwa*, previous molecular studies have identified three major hypotheses for the position of *Pucrasia*: 1) within “*Tragopan* and allies” (Crowe et al., 2006; Eo et al., 2009; Lucchini and Randi, 1999), 2) sister to turkey-grouse (Hosner et al., 2016a; Wang et al., 2013), and 3) sister to *Perdix*-Gallopheasant (Bao et al., 2010; Kimball et al., 2011; Kimball and Braun, 2008; Meiklejohn et al., 2014; Meng et al., 2008; Shen et al., 2010). This last hypothesis appears to be driven by signal in the mitochondrial genome (Wang et al., 2013) as we also found here.

For discordance among deeply diverged taxa, such as observed here, there are several possible causes. First, phylogenetic mis-estimation of either the mitochondrial or nuclear phylogeny (or both) may have occurred, such that the discordance is in the estimate(s) of phylogeny not in the underlying data. To obtain the best estimate of the mitochondrial phylogeny it is important to use complete mitogenomes (Meiklejohn et al., 2014) due to the existence of

conflict among estimates of phylogeny from different mitochondrial regions (Cox et al., 2007) and/or the low power of individual mitochondrial regions. However, analyses of mitochondrial data can be sensitive to model parameters (e.g., Braun and Kimball, 2002; Meiklejohn et al., 2014; Phillips et al., 2010; Powell et al., 2013). In the current study, we applied both DNA and codon models for protein coding regions in our mitogenome analyses, and also tried different programs (RAxML vs IQtree). Use of putatively better models (partitioning or codon models) did not resolve most conflicts between the mitochondrial and UCE data, although partitioning did result in agreement between the mitogenome and UCE data for *Rhizothera*. However, we note that the use of models that better accommodate the patterns of molecular evolution does not always result in the recovery of historical signal; this is certainly true for complete mitogenomes (e.g., Meiklejohn et al., 2014).

Improved taxon sampling can also improve phylogenetic estimation. Since several large-scale studies (Jetz et al., 2014; Burleigh et al., 2015; Stein et al. 2015) found a sister relationship between *Rhizothera* and *Pucrasia*, we hypothesized that the inclusion of *Rhizothera* might stabilize the position of *Pucrasia* given that even adding a single taxon that diverges close to an unresolved node of interest should facilitate phylogenetic resolution (Townsend and Lopez-Giraldez, 2010). However, the current study does not support the previous molecular hypotheses regarding *Rhizothera* and the position of *Pucrasia* still depends upon data type. Thus, while including more taxa to break long branches may be necessary to resolve some uncertainties, our inclusion of more taxa likely to affect the relationship of *Pucrasia* did not resolve conflicts in this instance (see also Meiklejohn et al., 2014).

Other causes of discordance can be due to incomplete lineage sorting or hybridization. We explored these two possibilities by reducing the UCE gene trees into rooted three taxon trees

and examining the percentage of each topology. Under this condition, discordance due to the multispecies coalescent is expected to yield a majority topology and two equiprobable minority topologies, while hybridization would be expected to lead to a majority topology and asymmetric minority topologies. For *Pucrasia*, we trimmed branches of the gene trees to only include *Pucrasia*, *Syrnaticus*, *Lagopus*, and *Rhynchortyx* (the last of which was used as the root). The most common gene tree did match the UCE topology (sister to turkey and grouse; 29.6% of trees) and support for the other two alternative topologies was equal (17.7 and 17.6%); the remaining 35% of gene trees were unresolved. For *Lerwa*, the rooted three taxon trees contained *Lerwa*, *Ithaginis*, *Syrnaticus* and rooted to *Rhynchortyx*. Again, we also found the UCE topology was most common (45.5% of trees), while the two alternative topologies were found in roughly equal proportions (15.9 and 15.0%; the remaining 23.6% of gene trees were unresolved). Thus, in both of these cases we have results consistent with lineage sorting, and inconsistent with hybridization.

One other explanation for discordance is due to selection that may drive convergence. Both *Ithaginis* and *Lerwa* are distributed at high altitudes, and it is possible that their mitogenomes have convergent adaptations to cold temperatures and lower partial pressures of oxygen. However, we did not identify any amino acid substitutions unique to both *Ithaginis* and *Lerwa* that would suggest convergence. Additionally, it is not clear what selective factor could drive the position of *Pucrasia*, and a simpler explanation for both cases may be that of incomplete lineage sorting.

Even if incomplete lineage sorting does explain the differences between the UCE and mitogenome topologies, we feel the robust relationship given by UCE data should be considered as the likely species tree. This is in part due to the observation that analyses that incorporated the

multispecies coalescent supported the UCE topology, suggesting neither conflicting relationship is in the anomaly zone (Degnan and Rosenberg, 2006; Liu and Edwards, 2009). Since mitochondrial genomes typically do not undergo recombination (e.g., Berlin and Ellegren, 2001; Berlin et al., 2004; but see Sammler et al., 2011), the mitogenome represents only a single locus whereas the large number of UCE loci provided a genome-wide sample of many different genes. Moreover, modeling the rapidly evolving mitogenome data is more challenging than that of nuclear data (different models return different results as shown in the current study; see also Meiklejohn et al., 2014). Additionally, UCEs exhibit much less homoplasy than mitochondrial data (McCormack et al., 2012; Meiklejohn et al., 2016). Thus, while understanding factors that may drive nuclear-mitochondrial differences may be of continued interest, our UCE data has likely provided an answer to the phylogenetic position of the phasianid taxa examined here.

4.3. Deep mitogenomic divergences raise the possibility of cryptic species

Of the six species where mitogenomes were available from two individuals, at least one of them demonstrated substantial divergence that could represent cryptic species (*Ithaginis*). With the exception of the two *Rhizothera* samples, no attempt was made to sample individuals from different populations (in those five cases, one mitogenome was published and one was collected through our sequence capture). Thus, there may be additional cases within species we examined where more strategic geographic sampling could have resulted in identification of deep divergences. The single case of deep divergence occurred in a highly polymorphic species, whereas in the other five species in which we examined the mitochondria showed little or no polymorphism across their species range. Deep mitogenomic divergence alone is insufficient to

split a named species but the observation of this divergence in a polytypic species is suggestive. However, it is important to note that there are a number of highly polymorphic species that also have relatively large numbers of defined subspecies within the Phasianidae overall. If these other species are like *Ithaginis* and show deep divergences, there may be many more species in the Phasianidae than are currently recognized.

Acknowledgements

We thank the museums and collectors that provided samples necessary for this study: Field Museum of Natural History (FMNH) and Yale Peabody Museum (YPM). We used computational resources provided by UF Research Computing (HiPerGator). Two anonymous reviewers, Scott Edwards and Lab members of Stephen Smith provided useful comments for revising the paper. This work was supported by the National Science Foundation DEB-1118823 to R.T.K. and E.L.B. N.W. and B.L. were supported by the National Natural Science Foundation of China grants 31360510 (N.W) and 31301894 (B.L.).

References

- Bao, X.K., Liu, N.F., Qu, J.Y., Wang, X.L., An, B., et al. 2010. The phylogenetic position and speciation dynamics of the genus *Perdix* (Phasianidae, Galliformes). *Mol. Phylogenet. Evol.* 56, 840–847.
- Berlin, S., Ellegren, H., 2001. Evolutionary genetics: Clonal inheritance of avian mitochondrial DNA. *Nature* 413, 37–38.
- Berlin, S., Smith, N.G.C., Ellegren, H., 2004. Do avian mitochondria recombine? *J. Mol. Evol.* 58, 163–167.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics*, btu170.
- Braun, E.L., Kimball, R.T., 2002. Examining basal avian divergences with mitochondrial sequences: Model complexity, taxon sampling, and sequence length. *Syst. Biol.* 51, 614–625.
- Burleigh, J.G., Kimball, R.T. and Braun, E.L., 2015. Building the avian tree of life using a large-scale, sparse supermatrix. *Mol. Phylogenet. Evol.* 84, 53–63.
- Cheng, T.H., 1978. *Fauna sinica. Aves. Vol 4: Galliformes*, pp. 182–186. Sciences Press, Beijing.
- Chernomor, O., von Haeseler, A., Minh, B.Q., 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.*, in press. DOI: 10.1093/sysbio/syw037.
- Clements, J.F., Schulenberg, T.S., Iliff, M.J., Roberson, D., Fredericks, T.A., Sullivan, B.L., Wood, C.L., 2015. The eBird/Clements checklist of birds of the world: v2015. <http://www.birds.cornell.edu/clementschecklist/download/>.

- Cox, W.A., Kimball, R.T., Braun, E.L., 2007. Phylogenetic position of the New World quail (Odontophoridae): Eight nuclear loci and three mitochondrial regions contradict morphology and the Sibley-Ahlquist tapestry. *Auk* 124, 71–84.
- Crowe, T.M., Crowe, A.A., 1985. The genus *Francolinus* as a model for avian evolution and biogeography in Africa: 1. Relationships among species. In: Proceedings of the International Symposium on African Vertebrates (Schuchmann, K.-L. Ed.): Systematics, Phylogeny and Evolutionary Ecology. Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn.
- Crowe, T.M., Bowie, R.C.K., Bloomer, P., Mandiwana, T.G., Hedderson, T.A.J., et al. 2006. Phylogenetics, biogeography and classification of, and character evolution in, gamebirds (Aves: Galliformes): Effects of character exclusion, data partitioning and missing data. *Cladistics* 22, 495–532.
- Davison, G.W.H., 1985. Avian spurs. *J. Zool., Lond.* 206, 353–366.
- Davison, G.W.H. 1999. Notes on the taxonomy of some Bornean birds. *Sarawak Mus. J.* 56, 289–299.
- Degnan, J.H., Rosenberg, N.A., 2006. Discordance of species trees with their most likely gene trees. *PLoS Genet.* 2, e68.
- Delacour, J., 1951. *The Pheasants of the World*. Country Life Limited, London.
- Desjardins, P., Morais, R., 1990. Sequence and gene organization of the chicken mitochondrial genome: a novel gene order in higher vertebrates. *J Mol. Biol.* 212, 599–634.
- Dyke, G.J., Gulas, B.E., Crowe, T.M., 2003. Suprageneric relationships of galliform birds (Aves, Galliformes): A cladistic analysis of morphological characters. *Zool. J Linn. Soc.* 137, 227–244.

- EO, S.H., BININDA-EMONDS, O.R.P., CARROLL, J.P., 2009. Phylogenetic supertree of the fowls (Galloanserae, Aves). *Zool. Scripta* 38, 465–481.
- EDWARDS, S.V., XI, Z., JANKE, A., FAIRCLOTH, B.C., MCCORMACK, J.E., GLENN, T.C., ZHONG, B., WU, S., LEMMON, E.M., LEMMON, A.R., LEACHÉ, A.D., 2016. Implementing and testing the multispecies coalescent model: A valuable paradigm for phylogenomics. *Mol. Phylogenet. Evol.* 94, 447–462.
- FAIRCLOTH, B.C., GLENN, T.C., 2012. Not all sequence tags are created equal: designing and validating sequence identification tags robust to indels. *PLoS One*. 8, e42543.
- FAIRCLOTH, B.C., MCCORMACK, J.E., CRAWFORD, N.G., HARVEY, M.G., BRUMFIELD, R.T., GLENN, T.C., 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Syst. Biol.* 61, 717–726.
- GILL, F., DONSKER, D., 2016. IOC World Bird List (v 6.2). doi : 10.14344/IOC.ML.6.2.
- GRABHERR, M.G., HAAS, B.J., YASSOUR, M., LEVIN, J.Z., THOMPSON, D.A., AMIT, I., ADICONIS, X., FAN, L., RAYCHOWDHURY, R., ZENG, Q., et al. 2011. Fulllength transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* 29, 644–652.
- GRAVES, G.R., BRAUN, M.J., 1992 Museums: storehouses of DNA? *Science*. 255, 1335-6.
- HILLIER, L.W., MILLER, W., BIRNEY, E., WARREN, W., HARDISON, R.C., et al. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432, 695–716.
- HOSNER, P.A., BRAUN, E.L. and R.T. Kimball. 2015. Tertiary land bridges and global cooling shaped colonization history and diversification of New World quail (Aves: Galliformes: Odontophoridae). *J. Biogeogr.* 42, 1883–1895.

- Hosner, P.A., Faircloth, B.C., Glenn, T.C., Braun, E.L. and Kimball, R.T., 2016a. Avoiding missing data biases in phylogenomic inference: An empirical study in the landfowl (Aves: Galliformes). *Mol. Biol. Evol.* 33, 1110–1125.
- Hosner, P.A., Braun, E.L., Kimball, R.T. 2016b. Rapid and recent diversification of curassows, guans, and chachalacas (Galliformes: Cracidae) out of Mesoamerica: phylogeny inferred from mitochondrial, intron, and ultraconserved element sequences. *Mol. Phylogenet. Evol.* 102, 320–330.
- Houde, P., Braun, M.J., 1988. Museum collections as a source of DNA for studies of avian phylogeny. *Auk* 105, 773–776.
- Howard, R., Moore, A., 1980. *A Complete Checklist of the Birds of the World*. Oxford University Press, New York.
- Jarvis, E.D., Mirarab, S., Aberer, A.J., Li, B., Houde, P., Li, C., 2014. Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science* 346, 1320–1331.
- Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K., Mooers, A.O., 2012. The global diversity of birds in space and time. *Nature* 491, 444–448.
- Johnsgard, P.A., 1986. *The Pheasants of the World*. New York: Oxford University Press.
- Johnsgard, P.A., 1988. *The Quails, Partridges, and Francolins of the World*. Oxford University Press, Oxford.
- Johnsgard, P.A., 1999. *The Pheasants of the World*, 2nd edn. Oxford University Press, Oxford. pp. 92–99.
- Katoh, K., Standley, D.M., 2013. MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780.

- Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic. Acids. Res.* 30, 3059–3066.
- Kimball, R.T., Braun, E.L., 2008. A multigene phylogeny of Galliformes supports a single origin of erectile ability in non-feathered facial traits. *J Avian Biol.* 39, 438–445.
- Kimball, R.T., Braun, E.L., 2014. Does more sequence data improve estimates of galliform phylogeny? Analyses of a rapid radiation using a complete data matrix. *PeerJ* 2, e361.
- Kimball, R.T., Mary, C.M.S., Braun, E.L., 2011. A macroevolutionary perspective on multiple sexual traits in the Phasianidae (Galliformes). *Int. J Evol. Biol.* 2011, 1–16.
- Lambert, S.M., Reeder, T.W. and Wiens, J.J., 2015. When do species-tree and concatenated estimates disagree? An empirical analysis with higher-level scincid lizard phylogeny. *Mol. Phylogenet. Evol* 82,146–155.
- Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* 29, 1695–1701.
- Liu, L., Edwards, S.V., 2009. Phylogenetic analysis in the anomaly zone. *Syst. Biol.* 58, 452–460.
- Lucchini, V., Randi, E., 1999. Molecular evolution of the mtDNA control-region in galliform birds. In: Adams, N.J., Slotow, R.H. (Eds.), *Proceedings of the 22 International Ornithological Congress*. Durban. Birdlife South Africa, Johannesburg, pp. 732–739.
- Maddison, W.P., Maddison, D.R., 2015. Mesquite: A modular system for evolutionary analysis v3.04. <http://mesquiteproject.org>.
- Madge, S., McGowan, P., 2002. *Pheasants, Partridges & Grouse: Including Buttonquails, Sandgrouse and Allies*. Princeton University Press, Princeton, NJ.

- McCormack, J.E., Faircloth, B.C., Crawford, N.G., Gowaty, P.A., Brumfield, R.T., Glenn, T.C., 2012. Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Res.* 22, 746–754.
- McCormack, J.E., Harvey, M.G., Faircloth, B.C., Crawford, N.G., Glenn, T.C., Brumfield, R.T. 2013. A phylogeny of birds based on over 1,500 loci collected by target enrichment and high-throughput sequencing. *PLoS One* 8, e54848
- McCormack, J.E., Tsai, W.L.E., Faircloth, B.C., 2015. Sequence capture of ultraconserved elements from bird museum specimens. *Mol. Ecol. Resour.* doi: 10.1111/1755-0998.12466
- Meiklejohn, K.A., Danielson, M.J., Faircloth, B.C., Glenn, T.C., Braun, E.L., Kimball, R.T., 2014. Incongruence among different mitochondrial regions: a case study using complete mitogenomes. *Mol. Phylogenet. Evol.* 78, 314–323.
- Meiklejohn, K.A., Faircloth, B.C., Glenn, T.C., Kimball, R.T., Braun, E.L., 2016. Analysis of a rapid evolutionary radiation using ultraconserved elements (UCEs): Evidence for a bias in some multi-species coalescent methods. *Syst. Biol.* <http://dx.doi.org/10.1093/sysbio/syw014>, in press.
- Meng, Y., Dai, B., Ran, J.H., Li, J., Yue, B.S., 2008. Phylogenetic position of the genus *Tetraophasis* (Aves, Galliformes, Phasianidae) as inferred from mitochondrial and nuclear sequences. *Biochem. Syst. Ecol.* 36, 626–637.
- Mey, E., 2006. Rätselhaftes Vorkommen zweier Federlingsarten (Insecta, Phthiraptera, Ischnocera) auf dem Haldenhun *Lerwa lerwa* (Galliformes, Phasianidae)? Pp. 55-71. In:

- Hartman, M. & J. Weipert (Hrsg.): Biodiversität und Naturaussatattung im Himalaya (Erfurt) II. 534 pp. + Taf. I-XII.
- Mindell, D.P., Sorenson, M.D., Dimcheff, D.E., 1998. An extra nucleotide is not translated in mitochondrial ND3 of some birds and turtles. *Mol. Biol. Evol.* 15, 1568–1571.
- Minh, B.Q., Nguyen, M.A.T., von Haeseler, A., 2013. Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol.* 30, 1188–1195.
- Mirarab, S., Warnow, T., 2015. ASTRAL-II: Coalescent -based species tree estimation with many hundreds of taxa and thousands of genes. *Bioinformatics* 31, i44–i52.
- Mirarab, S., Reaz, R., Bayzid, M.S., Zimmermann, T., Swenson, M.S., Warnow, T., 2014. ASTRAL: Genome-scale coalescent-based species tree estimation. *Bioinformatics* 30, i541–i548.
- Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* 32, 268–274.
- Nylander, J.A.A., 2004. MrAIC.pl. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Payne, R.B., Sorenson, M.D., 2002. Museum collections as sources of genetic data. *Bonn. Zool. Beitr.* 51, 97–104.
- Persons, N.W., Hosner, P.A., Meiklejohn, K.A., Braun, E.L., Kimball, R.T., 2016. Sorting out relationships among the grouse and ptarmigan using intron, mitochondrial, and ultra-conserved element sequences. *Mol. Phylogenet. Evol.* 98, 123–132.
- Patel, S., Kimball, R.T., Braun, E.L., 2013. Error in phylogenetic estimation for bushes in the tree of life. *J Phylogenet. Evol. Biol.* 1, 110.

- Phillips, M.J., Gibb, G.C., Crimp, E.A., Penny, D., 2010. Tinamous and moa flock together: Mitochondrial genome sequence analysis reveals independent losses of flight among ratites. *Syst. Biol.* 59, 90–107.
- Potapov, R.L., 2000. New information on the snow partridge *Lerwa lerwa* (Hodgson 1833) and its systematic position. *Bull. of the Br. Ornithol. Club* 120, 112–120.
- Powell, A.F.L.A., Barker, F.K., Lanyon, S.M., 2013. Empirical evaluation of partitioning schemes for phylogenetic analyses of mitogenomic data: an avian case study. *Mol. Phylogenet. Evol.* 66, 69–79.
- Rach, J., DeSalle, R., Sarkar, I.N., Schierwater, B., Hadrys, H., 2008. Character-based DNA barcoding allows discrimination of genera, species and populations in *Odonata*. *Proc. R. Soc. Lond. B. Biol. Sci.* 275, 237–247.
- Roure, B., Baurain, D., Philippe, H., 2013. Impact of missing data on phylogenies inferred from empirical phylogenomic data sets. *Mol. Biol. Evol.* 30, 197–214.
- Russell, R.D., Beckenbach, A.T., 2008. Recoding of translation in turtle mitochondrial genomes: programmed frameshift mutations and evidence of a modified genetic code. *J Mol. Evol.* 67, 682–695.
- Sammler, S., Bleidorn, C., Tiedemann, R., 2011. Full mitochondrial genome sequences of two endemic Philippine hornbill species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. *BMC Genomics* 12, 35.
- Sayyari, E., Mirarab, S., 2016. Fast coalescent-based computation of local branch support from quartet frequencies. *Mol. Biol. Evol.* msw079.

- Shen, Y.Y., Liang, L., Sun, Y.B., Yue, B.S., Yang, X.J., et al. 2010. A mitogenomic perspective on the ancient, rapid radiation in the Galliformes with an emphasis on the Phasianidae. *BMC Evol. Biol.* 10, 132.
- Shen, Y.Y., Dai, K., Cao, X., Murphy, R.W., Shen, X.J., Zhang, Y.P., 2014. The updated phylogenies of the Phasianidae based on combined data of nuclear and mitochondrial DNA. *PLoS ONE* 9, e95786.
- Smith, B.T., Harvey, M.G., Faircloth, B.C., Glenn, T.C., Brumfield, R.T. 2014. Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. *Syst. Biol.* 63, 83–95.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and postanalysis of large phylogenies. *Bioinformatics* 30, 1312–1313.
- Stein, R.W., Brown, J.W., Mooers, A.Ø., 2015. A molecular genetic time scale demonstrates Cretaceous origins and multiple diversification rate shifts within the order Galliformes (Aves). *Mol. Phylogenet. Evol.* 92, 155–164.
- Sun, K., Meiklejohn, K.A., Faircloth, B.C., Glenn, T.C., Braun, E.L., Kimball, R.T., 2014. The evolution of peafowl and other taxa with ocelli (eyespot): A phylogenomic approach. *Proc. R. Soc. Lond. B. Biol. Sci.* 281, 20140823.
- Swofford, D.L., 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, MA.
- Toews, D.P., Brelsford, A., 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Mol. Ecol.* 21, 3907–3930.
- Tonini, J., Moore, A., Stern, D., Shcheglovitova, M., Ortí, G., 2015. Concatenation and species tree methods exhibit statistically indistinguishable accuracy under a range of simulated

- conditions. PLoS Curr. Tree of Life doi: 10.1371/currents.tol.34260cc27551a34527b34124ec34265f36334b34266be.
- Townsend, J.P., Lopez-Giraldez, F., 2010. Optimal selection of gene and ingroup taxon sampling for resolving phylogenetic relationships. Syst. Biol. 59, 446–457.
- Wandeler, P., Hoeck, P.E., Keller, L.F., 2007. Back to the future: Museum specimens in population genetics. Trends Ecol. Evol. 22, 634–642.
- Wang, N., Kimball, R.T., Braun, E.L., Liang, B., Zhang, Z., 2013. Assessing phylogenetic relationships among Galliformes: A multigene phylogeny with expanded taxon sampling in Phasianidae. PLoS One 8, e64312.
- Wang, N., Kimball, R.T., Braun, E.L., Liang, B., Zhang, Z., 2016. Ancestral range reconstruction of Galliformes: The effects of topology and taxon sampling. J. Biogeogr. doi:10.1111/jbi.12782.
- Ward, R.D., 2009. DNA barcode divergence among species and genera of birds and fishes. Mol. Ecol. Resour. 9, 1077-1085.
- Yang, L., Wen, J.X., Yang, X.J., 1994. On the taxonomy of blood pheasant (*Ithaginis*). Zool. Res. 15, 21–30.
- Zeng, T., Tu, F., Ma, L., Yan, C., Yang, N., Zhang, X., Yue, B., Ran, J., 2013. Complete mitochondrial genome of blood pheasant (*Ithaginis cruentus*). Mitochondr. DNA 24, 484–486.
- Zhan, X., Zheng, Y., Wei, F., Bruford, M.W., Jia, C., 2011. Molecular evidence for Pleistocene refugia at the eastern edge of the Tibetan Plateau. Mol. Ecol. 20, 3014–3026.

Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. The University of Texas at Austin.

Table 1. divergences between individual mitogenome sequences within the same species.

		uncorrected <i>p</i> distance
<i>Argusianus argus</i>	<i>A. Argus</i> JQ713768	0.00329
<i>Ithaginis cruentus</i>	<i>I. cruentus</i> NC_018033	0.03024
<i>Perdix dauurica</i>	<i>P. dauurica</i> NC_020588	0.00086
<i>Rhizothera longirostris</i> Myanmar	<i>R. longirostris</i> Borneo	0.01549
<i>Tetrastes bonasia</i>	<i>T. Bonasia</i> NC_020591	0.00688
<i>Tetraogallus himalayensis</i>	<i>T. himalayensis</i> NC_027279	0.01060

Figure Legends:

Figure 1. Phylogenetic tree of 20 galliform taxa inferred with partitioned ML analysis from 3,692 concatenated UCE loci (<25% missing data).

Figure 2. Phylogenetic trees of 20 galliform taxa inferred with unpartitioned (A) and partitioned (B) ML analysis from 14,086 bp of mitochondrial genome data (both rRNAs and all 13 protein-coding genes). Bootstrap support was 100% unless indicated on the tree.

Figure 3. Phylogenetic trees of 25 galliform mitogenomes inferred with partitioned ML analysis from 14,088 bp of mitochondrial genome data (both rRNAs and all 13 protein-coding genes). Bootstrap support was 100% unless indicated on the tree.

Figure 8

A: MT unpartitioned trees

bootstrap support:

RAxML/IQtree

B: MT partitioned trees (DNA model)

bootstrap support:

RAxML/IQtree

Figure "