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

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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.

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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),

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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 3000 m to 5500 m, Cheng, 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 4817 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 [~700 bp 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., 2017), 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 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; Hosner et al., 2016b).

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.