

## PARTIAL CYTOCHROME *B* SEQUENCE OF CRESTED ARGUS FROM PENINSULAR MALAYSIA

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The Crested Argus (*Rheinardia ocellata*) is a large peafowl-like but elusive species with amazing plumage. It is rare in captivity and listed as Endangered on the IUCN Red List of Threatened Species (BirdLife International, 2018) and is listed in CITES Appendix 1. This species is totally protected under the Wildlife Conservation Act 2010. Two subspecies are recognised namely *Rheinardia ocellata ocellata* which can be found in Vietnam and Laos (Robson *et al.*, 1991; Thewlis *et al.*, 1998; Gray *et al.*, 2014) while *Rheinardia ocellata nigrescens* is endemic to Peninsular Malaysia (Mamat & Yasak, 1998; McGowan & Madge, 2010). *R. o. nigrescens* possess a few distinct morphological characteristics compared to *R. o. ocellata* (Wells, 1999; McGowan & Kirwan, 2020). *R. o. nigrescens* was first described as a subspecies by Rothschild (1902) based on three specimens (two males and one female) caught at Ulu Pahang, Pahang. Morphologically, *R. o. nigrescens* has darker colouration on its crest and plumage, and its ocellations is densely spaced compared to *R. o. ocellata* (Wells, 1999; McGowan & Madge, 2010). Besides, the supercilium and throat patch of *R. o. nigrescens* is buff in colour while the nominate subspecies are whitish (McGowan & Madge, 2010). Male *R. ocellata* has a long tail while the female is usually less than half the size of the male (WWF, 2005). Figure 1 shows an image

of a pair of *R. o. nigrescens* caught using camera-trapping at Hulu Dungun, Terengganu. Davison *et al.* (2020) recently proposed that *R. o. nigrescens* to be elevated from subspecies status and be classified as a distinct species from *R. o. ocellata* based on morphology and acoustic evidence.

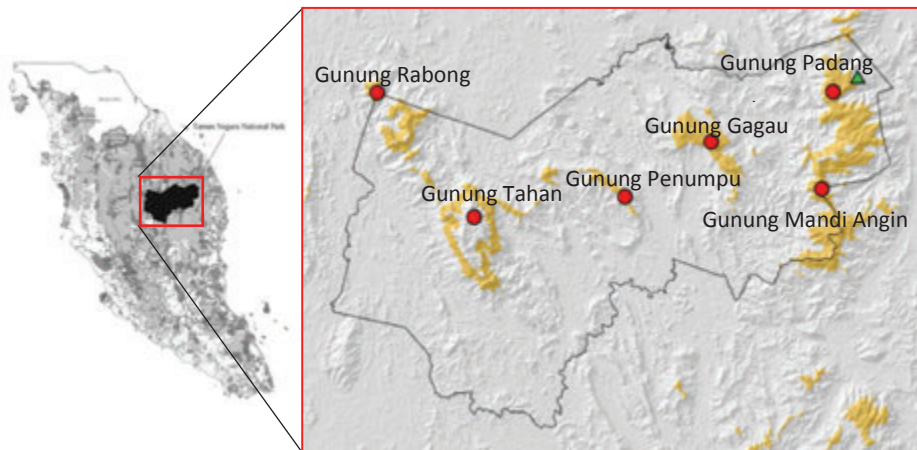
Distribution of *R. o. nigrescens* is confined to mountain range (Figure 2) on the east-central of Peninsular Malaysia and can be found at the altitudinal range between 800 to 1380 m above sea level (asl) (Mamat & Yasak, 1998; Liang *et al.*, 2018). In terms of conservation breeding effort, several attempts have been made previously by the Department of Wildlife and National Parks Peninsular Malaysia (PERHILITAN) to breed this species in captivity. In September 1988, a pair of *R. o. nigrescens* were captured at Gunung Rabung, Kelantan and were brought to Sungkai Wildlife Conservation Centre (SWCC) in Perak (Yatim, 1988). After several months living in captivity, the pair unfortunately died due to unknown causes (Othman, 1998). Another two male individuals were brought into SWCC but attempts to capture female individuals to pair with the males for breeding were unsuccessful. Eventually, the two males died after almost ten years in captivity. At present, the efforts to capture *R. o. nigrescens* for breeding purpose is still ongoing by PERHILITAN.

During a patrol called ‘Boots on the Ground’ conducted by PERHILITAN in 2019 to combat poachers in Taman Negara Terengganu, a carcass of *R. o. nigrescens* was found indiscriminately snared at Gunung Padang in Taman Negara Terengganu (Figure 2). The carcass was discovered at 932 m asl, which is within the altitudinal range of the species (Liang *et al.*, 2018). Feather sample from the carcass was brought back to the lab for the morphological reference collection. Prior to this, there was no biomaterial sample of *R. o. nigrescens* collected from Peninsular Malaysia. Besides, the validity of the taxonomic status of the two subspecies of *R. ocellata* has not been assessed using molecular data. Hence, it is essential to collect and utilise this valuable sample to obtain the genetic sequence to document the faunal diversity of Malaysia as well as for future reference.

Feather samples (Figure 3) were morphologically identified as *R. ocellata* by an avifauna expert from PERHILITAN based on colour and pattern. These samples were deposited in Wildlife Genetic Resource Bank (WGRB) of PERHILITAN with reference number OLT01. Genomic DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN, Germany) following the manufacturer’s protocol.



**Figure 1** Camera-trap image of a pair of *R. o. nigrescens* captured at Sungai Kelmin, Hulu Dungun, Terengganu in 2017.



**Figure 2** Map showing the locations of previous sighting records of *R. o. nigrescens* (red dots) in Taman Negara National Park. The location where the carcass was found is marked by the green triangle. Yellow shaded areas are the altitudinal range of the species (800 – 1380 m asl).



**Figure 3** Feather samples collected during the patrol.

Extracted DNA was amplified using cytochrome *b* (*cytb*) primers (Kocher *et al.*, 1989) of the mitochondrial DNA using the following PCR profile: pre-denaturation 94°C for 3 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 49°C for 30 s, and extension at 72°C for 40 s before a final extension at 72°C for 5 min after the last cycle for complete synthesis of DNA molecules. Post-PCR purification was done using Illustra Exo-ProStar (GE Healthcare, UK) and sequenced on an ABI3730 Genetic Analyzer (Applied Biosystems, USA).

The partial *cytb* sequence obtained in this study is 307-basepairs in length and was deposited in GenBank with the accession number MK907492. This sequence is 100% identical to *R. ocellata* in GenBank (Accession No. AF330060). The origin of this reference sample AF330060 (Kimball *et al.*, 2001) is however not mentioned by the depositor.

The sequences obtained were analysed and aligned with other Phasianidae sequences retrieved from GenBank (Table 1) using Geneious Prime (Biomatters, New Zealand). Table 2 showed the genetic distances calculated among the pheasant's species using Kimura-2 parameter model (K2P) (Kimura, 1980) as calculated in MEGA X (Kumar *et al.*, 2018). The genetic distances of *R. ocellata* as compared to the other pheasants ranged from 5.7 – 17.3%. Johns and Avise (1998) found an average of 3.5% of genetic distances for congeneric species in birds using K2P on *cytb*. Thus, a genetic distance of at least 3.5% can be considered as different species. Although *R. o. nigrescens* has been proposed to be elevated to a species status (Davison *et al.*, 2020), our current data indicate that there is no genetic difference between the two crested argus subspecies. Nonetheless, this finding cannot be confirmed until further genetic study can be carried out using more specimens as well as using other genetic segments.

**Table 1** Reference sequences retrieved from GenBank.

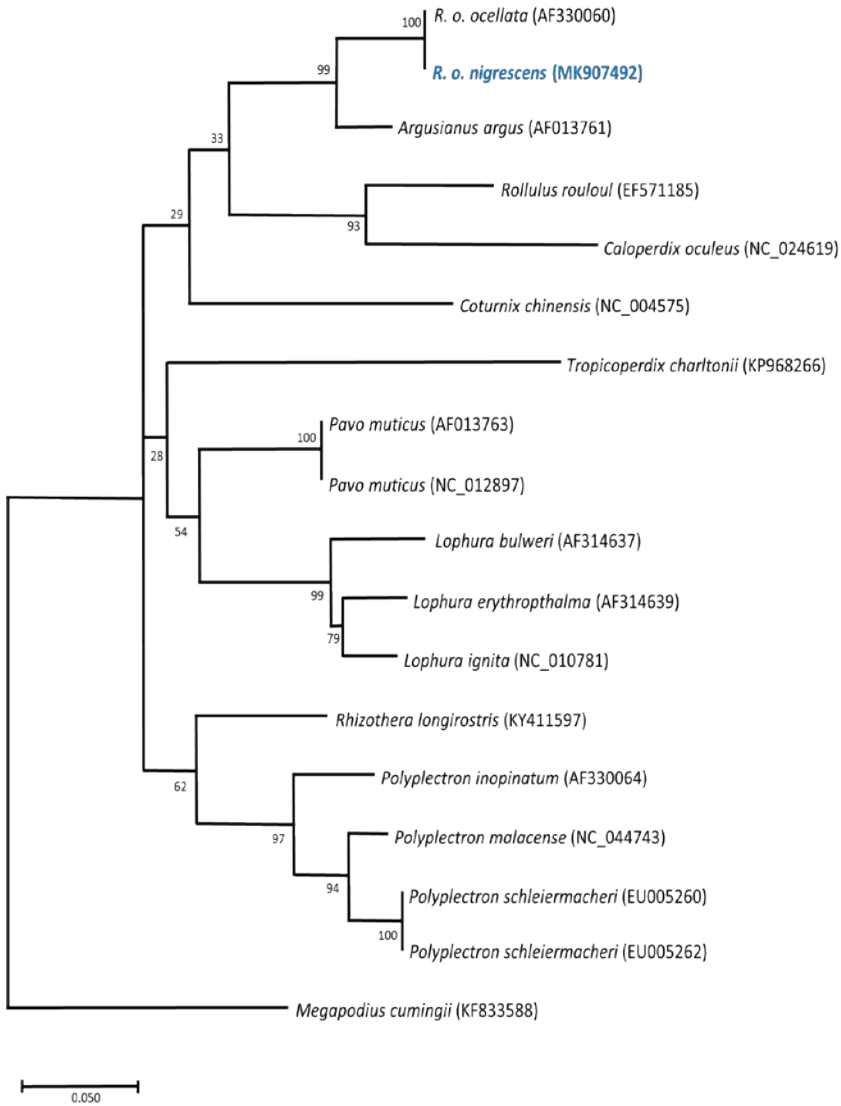
No.	Scientific name	Species name	GenBank Acc. No.
1	<i>Argusianus argus</i>	Great argus	AF013761
2	<i>Caloperdix oculeus</i>	Ferruginous partridge	NC_024619
3	<i>Coturnix chinensis</i>	Blue-breasted quail	NC_004575
4	<i>Lophura bulweri</i>	Bulwer's pheasant	AF314637
5	<i>Lophura erythrophthalma</i>	Crestless fireback	AF314639
6	<i>Lophura ignita</i>	Crested fireback	NC_010781
7	<i>Pavo muticus</i>	Green peafowl	AF013763, NC_012897
8	<i>Polyplectron inopinatum</i>	Mountain peacock-pheasant	AF330064
9	<i>Polyplectron malacense</i>	Malayan peacock-pheasant	NC_044743
10	<i>Polyplectron schleiermacheri</i>	Bornean peacock-pheasant	EU005260, EU005262
11	<i>Rheinardia ocellata</i>	Crested argus	AF330060
12	<i>Rhizothera longirostris</i>	Long-billed partridge	KY411597
13	<i>Rollulus rouloul</i>	Crested partridge	EF571185
14	<i>Tropicoperdix charltonii</i>	Chestnut-necklaced partridge	KP968266

Phylogenetic tree was also constructed on MEGA X using maximum likelihood method and Hasegawa-Kishino-Yano model with 1000 bootstrap replication (Felsenstein, 1985). The Philippine scrubfowl (*Megapodius cumingii*) from Family Megapodiidae (Order Galliformes) was used as outgroup (GenBank Accession number KF833588). Phylogenetic tree (Figure 4) constructed using maximum likelihood method shows that *R. ocellata* is closely related to *Argusianus argus* (Great Argus). However, due to the short sequence, the validity of the taxonomic status between the two subspecies of *R. ocellata* cannot be confirmed. Nonetheless, the sequence generated in this study can be useful as a genetic reference of this elusive pheasant from Peninsular Malaysia.

More sample representatives and further molecular research using other molecular segments such as the complete mitochondrial genome (mitogenome) and Y-chromosome would be helpful to provide insight into the population genetics in order to formulate a comprehensive conservation and management plan for this spectacular species.

**Table 2** Genetic distances among the species within Phasianidae sequences.

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	<i>Rheinardia ocellata</i>														
2	<i>Argusianus argus</i>	5.7													
3	<i>Calopdix oculeus</i>	16.0	16.1												
4	<i>Coturnix chinensis</i>	15.5	15.1	18.1											
5	<i>Lophura bulweri</i>	16.9	14.3	17.8	16.4										
6	<i>Lophura erythrophthalma</i>	17.0	13.5	21.5	15.6	7.3									
7	<i>Lophura ignita</i>	17.0	14.3	20.1	18.1	17.8	4.6								
8	<i>Polyplectron malacense</i>	15.1	12.6	15.9	13.4	12.6	13.5	14.4							
9	<i>Polyplectron inopinatum</i>	14.3	14.3	17.6	14.7	14.7	14.8	14.8	6.9						
10	<i>Polyplectron schleiermachersi</i>	15.2	12.6	16.8	13.8	13.8	13.9	14.7	3.9	7.7					
11	<i>Pavo muticus</i>	11.9	13.5	17.0	15.1	12.6	10.6	11.0	14.3	13.0	13.4				
12	<i>Rhizothera longirostris</i>	15.3	12.7	17.4	15.1	12.3	11.9	12.8	9.5	9.9	10.6	10.7			
13	<i>Rollulus rouloul</i>	13.5	13.5	13.1	15.9	19.6	17.8	19.7	16.0	14.7	16.0	13.1	13.9		
14	<i>Tropicoperdix charltonii</i>	17.0	15.2	19.2	17.3	15.6	17.0	16.6	17.3	17.3	17.3	14.8	16.5	18.7	
15	<i>Megapodius cumingii</i>	17.3	16.0	23.3	20.8	19.6	18.6	20.0	16.0	17.7	18.1	17.3	17.8	19.9	23.0



**Figure 4** Phylogenetic tree constructed using maximum likelihood method and Hasegawa-Kishino-Yano model with 1000 bootstrap replication based on partial *cytb* sequences (307-bp) of Crested Argus (MK907492) and other Phasianidae species.



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## REFERENCES

- BirdLife International (2018). *Rheinardia ocellata*. The IUCN Red List of Threatened Species 2018:e.T22679412A132430734.<https://dx.doi.org/10.2305/IUCN.UK.20182.RLTS.T22679412A132430734.en>. Downloaded on 29 March 2020.
- Davison, G.W.H., Boesman, P., Collar, N.J. & Puan, C.L. (2020). Species rank for *Rheinardia ocellata nigrescens* (Phasianidae). *Bulletin of the British Ornithologists' Club*, **140**(2): 182-194.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**(4): 783-791.
- Gray, T.N., Quang, H.A.N. & Van, T.N. (2014). Bayesian occupancy monitoring for Annamite endemic biodiversity in central Vietnam. *Biodiversity Conservation*, **23**: 1541-1550.
- Johns, G.C. & Avise, J.C. (1998). A comparative summary of genetic distances in the vertebrates from the mitochondrial *cytochrome b* gene. *Molecular Biology and Evolution*, **15**(11): 1481-1490.
- Kimball, R.T., Braun, E.L., Ligon, J.D., Lucchini, V. & Randi, E (2001). A molecular phylogeny of the peacock-pheasants (Galliformes: *Polyplectron* spp.) indicates loss and reduction of ornamental traits and display behaviors. *Biological Journal of the Linnean Society*, **73**: 187-198.
- Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**(2): 111-120.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X. & Wilson, A.C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA*, **86**(16): 6196-6200.

- Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**: 1547-1549.
- Liang, S.H., Yong, D.L., Abdul Hashim, A.K., Abdul Patah, P., Ilias, R., Abdul Halim, H.R., Mohd. Kharip Shah, A.K., Le, T.T. & Clements, G.R. (2018). Peninsular Malaysia's forgotten pheasant: recent records and distribution of the Crested Argus *Rheinardia ocellata*. *Forktail*, **34**: 48-51.
- Mamat, I.H. & Yasak, M.N. (1998). The status and current distribution of the Crested Argus *Rheinardia ocellata nigrescens* in Peninsular Malaysia. *Bird Conservation International*, **8**: 325-330.
- McGowan, P.J.K. & Kirwan, G.M. (2020). Crested argus (*Rheinardia ocellata*), version 1.0. In *Birds of the world* (del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E., eds.), Cornell Lab of Ornithology, Ithaca, New York, USA. Accessed at <https://doi.org/10.2173/bow.crearg1.01>. (Version on 06 July 2020).
- McGowan, P.J.K. & Madge, S. (2010). *Pheasants, partridges and grouse, including buttonquails, sandgrouse and allies*. London: A. & C. Black Publishers Ltd.
- Othman, H.S. (1998). Galliformes captive breeding and management - DWNP's experience. *The Journal of Wildlife and Parks*, **16**: 1-10.
- Robson, C.R., Eames, J.C., Newman, M., Nguyen Cu & Truong Van La. (1991). Forest bird surveys in Vietnam 1989/1990: final report. Unpublished report to the International Council for Bird Preservation.
- Rothschild, W. (1902). A new subspecies of *Rheinardius ocellatus*. *Bulletin of the British Ornithologists' Club*, **12**: 55-56.
- Thewlis, R.M., Timmins, R.J., Evans, T.D. & Duckworth, J.W. (1998). The conservation status of birds in Laos: a review of key species. *Bird Conservation International*, **8**(S1): 1-159.
- Wells, D.R. (1999). The birds of the Thai-Malay Peninsula, vol. 1. London: Academic Press.
- World Wildlife Fund for Nature (WWF). (2005). Introducing the Crested Argus (*Rheinardius ocellata*). World Wildlife Fund for Nature. Accessed at <https://wwf.panda.org/?22878/introducing-the-crested-argus-irheinardia-ocellatai>. (Version on 06 July 2020).
- Yatim, S.H. (1988). Kuang Gunung. *Majalah PERHILITAN*, **8**(1): 2-9.