### **Reviewer Report**

Title: De novo assembly of the Indian Blue Peacock (Pavo cristatus) genome using Oxford Nanopore Technology and Illumina sequencing

**Version: Original Submission Date:** 8/27/2018

**Reviewer name: Daniel Ence** 

#### **Reviewer Comments to Author:**

The pdf file that I received included two copies of the manuscript text. The second copy begins on page 30 of the pdf file and appears to be identical to the first. I restricted my comments and review to the first copy of the manuscript.

The manuscript is much improved in most aspects. The introduction and conclusion are much improved although the discussion of sexual selection still needs to be reduced. The analysis of Gene Ontology (GO) terms and gene homology across birds are incorrect though. Gene ontology terms are divided into three domains, Biological Process, Cell Component, and Molecular Function. These domains are separate from each other in GO terms but the manuscript (in the results and discussion sections and the supplementary Fig3a-c) treat them as if one gene can only be in one of those three domains. Most analyses just focus on one of those three domains, usually Biological Process or Molecular function. The claims of overrepresented gene ontology terms aren't tied to any comparisons that I can see in the manuscript, so it isn't clear how any such claim can be made. Claims of overrepresentation should also be backed up by statistical tests for significance. Either the claims of overrepresentation should be removed or analysis should be presented to backup those claims. There are tools that work with GO term annotation to identify overrepresented terms, account for the hierarchy, and test for statistical significance. Two such tools are ClueGO and BinGO which are plugins to the Cytoscape tool. The mere presence of FGF and KIT proteins in the peacock genome isn't surprising since they are probably present in a large number of other animals' genomes too. Some analysis (even a count of homologs, or some sort of comparative alignment analysis) should be presented. The results in the "Avian Protein Family" section also need to be re-examined, specifically the finding that ~15,000 out of the ~23,000 peacock genes have no ortholog in other bird genomes. Either a majority of peacock genes are misannotations of some kind, or the CD-HIT clustering was too stringent to identify their homologs.

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