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Original article

Molecular identification of *Campanulotes bidentatus* Scopoli, 1763 (Phthiraptera, Philopteridae) infecting the domestic pigeon *Columba livia* from Saudi Arabia

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ABSTRACT

The taxonomy of the order Phthiraptera is unstable and still problematic to researchers. Most of the current taxon classifications are mainly based on morphological features. *Campanulotes bidentatus* belongs to the chewing lice of the Philopteridae family that mostly parasitic on birds. There is a lack of sequence data and phylogenetic analyses on the family Philopteridae. In the current study, *C. bidentatus* was collected from the domestic pigeon *Columba livia* and identified morphologically and molecularly based on the mitochondrial cytochrome *c* oxidase subunit 1 gene (*COI*). The infection rate of the *Campanulotes* genus was approximately 58.82% in this study. Phylogenetic analysis based on the mt *COI* gene was informative for members of Philopteridae and the group taxon genera formed distinct clades. Future studies were recommended using the *16s rRNA* to enhance the tree topology and obtain clear differentiation between genera.

1. Introduction

The order Phthiraptera Haeckel, 1896 includes a large group of ectoparasites, including chewing lice. They are not in a symbiotic relationship with their host; instead, it is well known the negative effect of these ectoparasites on the hosts productivity and vitality (Jahantigh et al., 2016). Besides, these ectoparasites are of clinical importance when they can transmit infectious and allergic diseases to humans (James, 2015). Also, these chewing lice infect most families of birds (Saxena et al., 2007; Beg et al., 2008; Khan et al., 2008; Kumar and Hasan, 2015; Naz et al., 2016). Of these birds, domestic pigeons (*Columba livia*) are heavily infected with avian lice belonging to the genus *Campanulotes* (Clayton and Walther, 2001; Khan et al., 2009). The degree of infection is controlled by several factors, such as age, sex, breed, and environmental factors (Nadeem et al., 2007).

The population of the genus *Campanulotes* Kéler, 1939 is usually identified morphologically based on the chaetotaxy, the intensity of sclerotization, and the body size (Złotorzycka et al. (1974); Mey et al. (1994)). For example, *Campanulotes defectus* Tendeiro, 1969 can be differentiated from *Campanulotes flavus* Rudow, 1869 based on the antennal shape and chaetotaxy of the female subgenital plate (Tendeiro and Estudos, 1969). Besides, larval

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instars of *Campanulotes bidentatus* Scopoli, 1763 can be distinguished by their setal patterns (Kayß et al., 2006). Several specimens of *C. bidentatus* have been compared. However, morphological-based identification is mostly misleading, particularly when distinguishing between genera at the same level.

To the best of our knowledge, no studies are describing the molecular identification of the species *C. bidentatus* in Saudi Arabia. Furthermore, very few sequences from this genus have been deposited in the GenBank database. The whole mitochondrial genome of *C. bidentatus* was sequenced in a previous study, revealing a circular genome of 14,804 bp (Covacin et al., 2006). The mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) and the nuclear Elongation Factor 1 alpha (*EF1α*) genes were used for molecular systematics studies of avian feather lice (Johnson et al., 2001). Sequences of the *COI* are more divergent, and *COI*-based phylogenetic analysis is more informative than EF1α-based analysis. Due to its high inter- and intra-species variation, *COI* has been used previously to study variations among the head lice *Pediculus humanus corporis* (Boutellis et al., 2014). In other studies, *COI* was also used to investigate the evolutionary histories of chewing lice of gophers (Hafner et al., 1994; Demastes et al., 2012).

In Saudi Arabia, there is a deficiency in studies relating to pigeon ectoparasites and their taxonomy, the purpose of this study is to establish the use of the mitochondrial gene in the identification of *C. bidentatus* infecting pigeons and forming their association with other globally dispersed lice of the same genus.

2. Materials and methods

2.1. Ectoparasites collection and morphological identification

The domestic pigeons (*Columba livia*, 34 in total with the same age and weight) were purchased from a commercial poultry farm in Shaqraa Province, Riyadh, Saudi Arabia. Pigeons were transported immediately to the animal house at Zoology Department, College of Science. Less-attached ectoparasites were collected by brushing the plumage into a white tray. The whole body of each pigeon was carefully examined with the help of a magnifying glass. Attached ectoparasites were gently removed with a pair of forceps, counted, and transferred to labeled vials with absolute ethanol, and preserved for further molecular identification. The infection rate of examined pigeons by lice was calculated according to Nisham and Adesh (2018). Morphological identification was achieved by following standard methods of Cheesbrough (1991) using a stereomicroscope provided with a digital camera (Nikon SMZ18, Japan) at a magnification power of 1×.

2.2. Molecular identification

For DNA extraction, lice preserved specimens were rinsed three times with 1× phosphate-buffered saline (PBS) to remove ethanol. Two to five lice were cut longitudinally into two parts, homogenized in 180 μL ATL buffer, and treated with proteinase K for 16 h in a water bath at 56 °C. DNA was then extracted according to the manufacturer's instructions using a QIAamp® DNA Mini Kit (QIAGEN, Germany). The extracted DNA was used to amplify the mt *COI* gene using the primer set (LCO1490_F) (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and (HCO2198_R) (5′-GTA AAT ATA TGR TGD GCTC-3′) mentioned by Folmer et al. (1994). Amplified PCR products were then purified using Illustra GFX PCR DNA and a Gel Band Purification Kit (GE Healthcare Dharmacon, Inc.). DNA sequencing of the purified fragments was achieved on both strands using a 3130 × Genetic Analyzer (Biosystems, Thermo Fisher Scientific, USA). The raw sequences were edited and assembled using Bioedit software ver. 7.2.5 (Hall, 1999). The BLAST tool was used to identify the assembled sequences. To investigate the genetic relatedness between the different genera and species, a phylogenetic tree was constructed using the character-based maximum-likelihood (ML) method in MEGA ver. 7.0 (Kumar et al., 2016). To test the reliability, bootstrapping was conducted with 1000 replicates (Felsenstein, 1985). Besides, sequences of some out-groups taxon genera were included phylogenetic analysis.

3. Results

A total of 44 lice were collected from 21 of the 34 pigeons examined (an infection rate of 58.82%, ~1 louse per host) and identified morphologically as the third larval instar of *Campanulotes bidentatus*. It is characterized by a well-sclerotized body, two marginal temporal setae, distinct external segmentation of the abdomen, and the setal pattern of the lateral margin of the mesometanotum (Fig. 1). The phylogeny based on the mt *COI* gene sequence was performed to investigate the recovered phthirapteran species' exact taxonomic position. Only one species was belonging to the family Philopteridae and identified as *Campanulotes bidentatus*. The obtained sequences were deposited in the GenBank with accession numbers (no. of nucleotides, GC content) as following: MK936058.1 (544 nt, 35.8%), MK936059.1 (563 nt, 35.9%), MK936060.1 (563 nt, 35.9%), MK936061.1 (642 nt, 35.8%), and to MK936062.1 (585 nt, 36.1%). The current phylogeny based on 41 taxa and the alignment of those sequences demonstrated minimal divergence among populations of *C. bidentatus* and other comparable genera (Fig. 2). The present dendrogram was consisted of two clades, the first one including taxa within Philopteridae distributed along two subclades: the former one clustered chewing lice genera of *Campanulotes*, *Physconelloides*, *Goniodes*, *Coloceras*, and *Goniocotes*, and the second subclade clustered four genera of *Auricotes*, *Kodocephalon*, and *Columbicola*. The second clade clustered out-groups species of the genera *Grylloidea*, *Kempiola*, *Oncerotrachelus*, *Otostigmus*, and *Pothea*. The ML tree showed a well-resolved distinct clade for the recovered ischnoceran species' with other members of Philopteridae species, especially those belonging to the genus *Campanulotes* with a bootstrapping value of 77% (Fig. 3). However, the *Campanulotes* strains collected from Saudi Arabia forming a separate clade with supporting value of 67%, indicating the high degree of similarity between these strains. In addition, some of the relationships among the lice of Columbiformes were relatively well supported by bootstrapping, including sister relationships (99%) between *Goniodes biordinatus* and *Physconelloides eurysema* and all of the body lice of Columbiformes.

4. Discussion

Taxonomy of the order Phthiraptera is unstable, and data about more morphological and/or molecular features are needed to enable clear demarcation between families, genera, and species. Four suborders have been recognized, of which the suborder Ischnocera comprises nearly 60% of all described louse species (Johnson et al., 2001). In the present study, chewing lice were observed in the domestic pigeons with higher infection rates, which consistent with other studies for estimation of different louse genera. Some louse genera have high infection rates, as *Columbicola tschulyschman* (141 lice/host), *C. bidentatus compar* (45.5 lice/host), *Colpocephalum turbinatum* (182.5 lice/host), and *Hohorstiella lata* (18.93 lice/host) (Singh et al., 1998). For *C. bidentatus*, a previous study by Dhoundiyal (2018) reported an infection rate of 80.14%. Other genera have low infection rates, such as *Brueelia vulgate* (3 lice/host) (Woodman and Dicke, 1954). The taxonomy of families and genera within Ischnocera is a subject of debate among researchers (Smith, 2000). Currently four families are recognised but one of these, the Philopteridae, is almost certainly paraphyletic. The generic-level taxonomy within Philopteridae has fluctuated, and a variety of gen-

Fig. 1. Third larval instar of *Campanulotes bidentatus* as viewed under a stereomicroscope with a digital camera (Nikon SMZ18) at a magnification power of 1×.

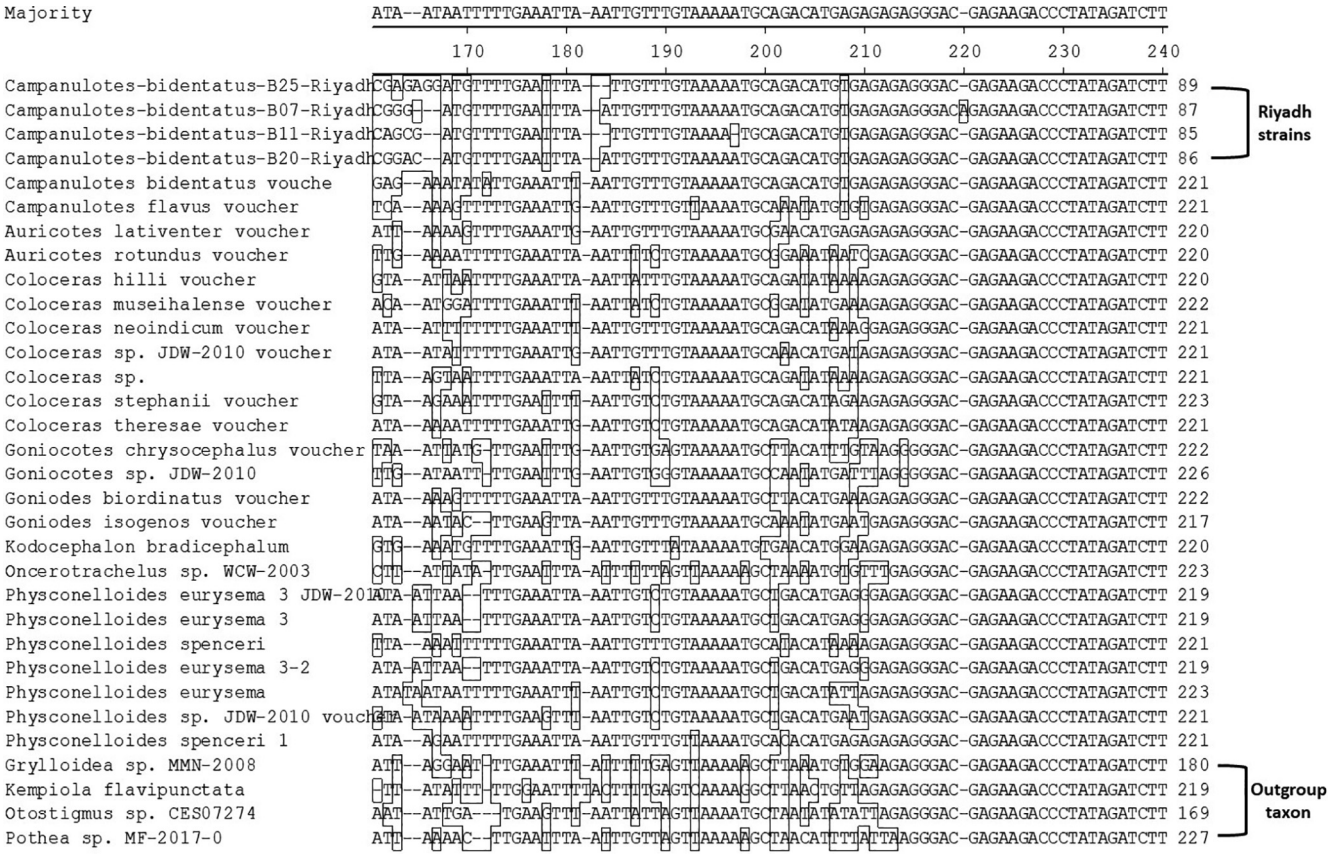

Fig. 2. Intraspecific variations based on the mt *COI* gene among populations of *C. bidentatus* and other genera of Ischnocera.

era have been recognized (Smith, 2001). The identification of the current chewing louse according to the morphological criteria of Kayß et al. (2006) was performed on the basis of the setal pattern of the tempora and the mesometathorax, and considered as the third larval instar of *C. bidentatus*. Morphological-based identification is mostly misleading, particularly when distinguishing between genera at the same level. Data regarding the sequence and phylogenetic analysis of the family Philopteridae are scarce (Covacin et al., 2006). In the present study, the confirmation study of the chewing louse is based on the mt *COI* gene. The phylogenetic dendrogram showed herein a clear separation of *Campanulotes* strains that recovered from Saudi Arabia, this finding refers to the difference between *Campanulotes* strains isolated from Saudi Arabia and other countries which could be attributed to environmental conditions, which is consistent with Schreiber et al. (2015). Herein, *Campanulotes flavus* is clearly separated from other species in the *Campanulotes* genus, which is consistent with Tendeiro's interpretation in 1971 that a subgroup of *Campanulotes* distributed on Australian phabine doves, is separated from other *Campanulotes*. In addition, Nasser et al. (2020) reported that the two genera of *Gonio-*

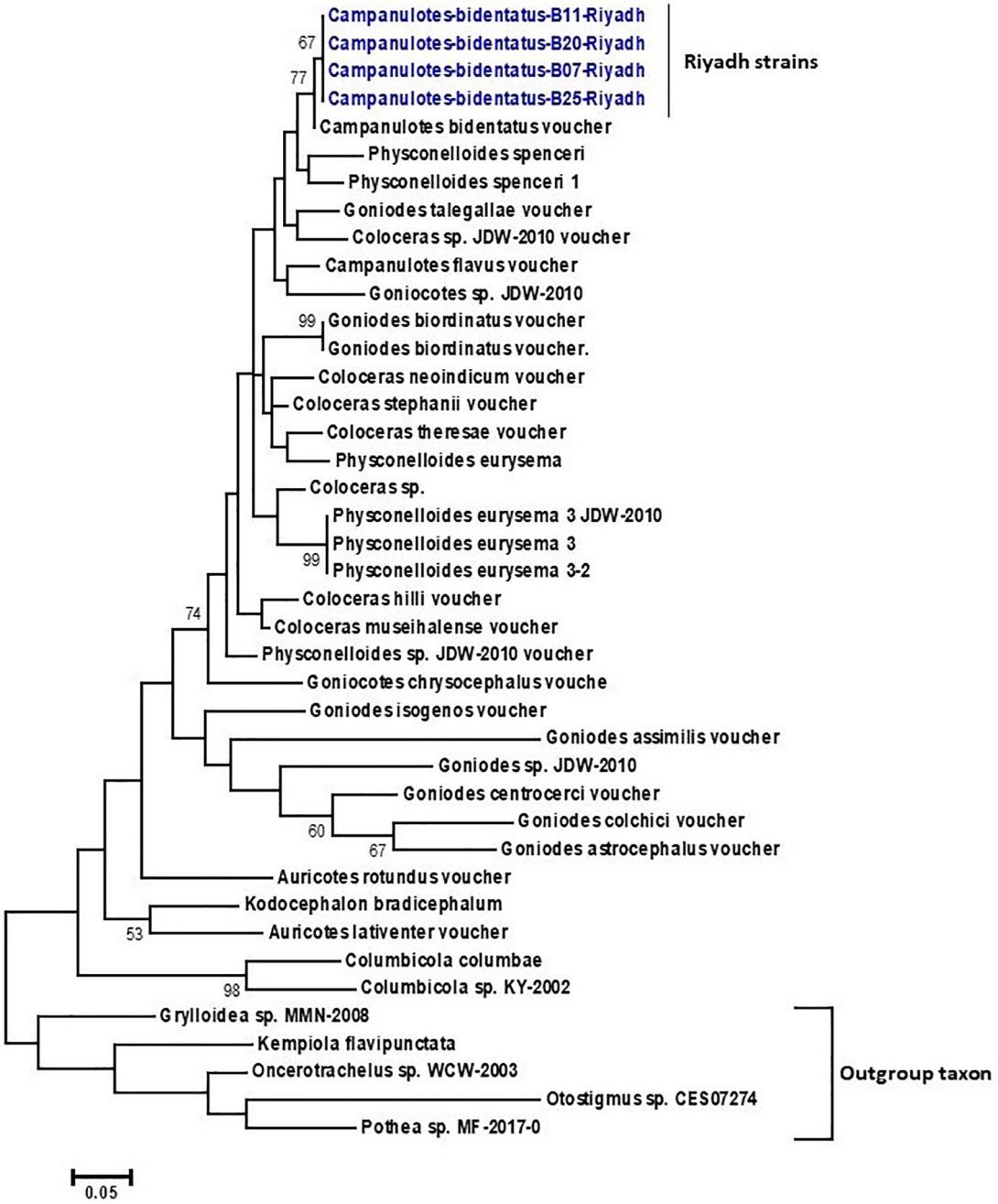

Fig. 3. Phylogenetic analysis based on the ML for the mt *COI* gene. Numbers above nodes refer to the bootstrapping values and those less than 50% were omitted from the tree.

cotes and *Goniodes* form a monophyletic group, while, this cluster acted as paraphyletic in the presence of the *Campanulotes* genus, which agreed with data presented herein.

Therefore, the mitochondrial *COI* gene is a useful tool in the molecular identification of louse species providing unique features that differentiated them from other global strains. Further studies should be included other genes as *16s rRNA* or a combination of different molecular markers, such as *COI, COII*, and *EF1α* genes, to enhance the tree topology and to obtain clear differentiation between families and genera.

Disclosure of potential conflicts of interest

The author has indicated that she has no conflict of interest regarding the content of this article. All other authors declare no competing interests.

Significance

The present study provided data about the usage of '*mt COI*' gene for identification of the avian lice, *Campanulotes bidentatus*, and confirms its evolutionary status in Philopteridae.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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