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CRITICAL COMMENT . . . . . .

A MISIDENTIFICATION CRISIS PLAGUES SPECIMEN-BASED RESEARCH: A CASE FOR GUIDELINES WITH A RECENT EXAMPLE (ALI ET AL., 2020)

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KEY WORDS

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ABSTRACT

A recent paper in this journal concerning parasites of rock pigeons (*Columba livia*) published by Ali and colleagues exemplifies a growing trend of misidentified parasites in the literature, despite increased online resources that should help facilitate accurate identification. In the Ali et al. paper, a pigeon louse in the genus *Columbicola* (Phthiraptera: Ischnocera) is misidentified as *Menopon gallinae*, which is a parasite of chickens (*Gallus gallus*) and their relatives; moreover, this louse is from an entirely different suborder of lice (Phthiraptera: Amblycera). Another louse is misidentified as *Goniodes dissimilis*, another parasite of chickens and junglefowl. In addition, photographs of cestodes from pigeons in the same paper are not sufficient to confirm identification. Misidentifications are fueled, in part, by increasing pressure to publish coupled with a decrease in taxonomic expertise. We consider the downstream consequences of misidentification and suggest guidelines for authors, reviewers, and editors that could help to improve the reliability of specimen-based research.

Ali et al. (2020) reported on ecto- and endo-parasites they collected from rock pigeons (*Columba livia* Gmelin, 1789) in Medina, Saudi Arabia. To the authors' credit, they included photographs of some of the parasites, allowing independent assessment. Unfortunately, the lice in the photograph are misidentified, even to the level of suborder, and the cestode images are not of sufficient quality to support identification. These errors are part of a growing trend in which investigators "identify" parasites from published host records, rather than by preparing and examining good quality specimens, photographs, and/or molecular markers of the parasites in question. The problem is exacerbated when investigators rely on outdated lists or lists of parasites from distantly related hosts.

In their paper, Ali et al. (2020, p. 722) state that, "Ectoparasites were identified according to Hutson (1984), Price et al. (2003), and Mansur et al. (2019)." Hutson (1984) could have been used to correctly identify the pigeon flies (*Pseudolynchia canariensis* (Macquart 1840)). However, the drawings and keys in Price et al. (2003), which are restricted to the level of genus and above, could not have been used to identify the species of lice. Ali et al. presumably used Mansur et al. (2019), yet this paper is restricted to lice from chickens, which belong to an unrelated host order (Galliformes instead of Columbiformes). It is unusual for lice of the same species to be found on more than 1 host family, let alone more than 1 host order. Lice are among the most host-specific parasites known (Price et al., 2003; Clayton et al., 2015); consequently, they provide an opportunity to explore the misidentification process in more detail.

Ali et al. misidentified the lice in figure 3J as *Menopon gallinae* (L., 1758), which is a common species found on chickens (*Gallus gallus* (L., 1758)) and a dozen other members of the galliform family Phasianidae (Price et al., 2003). The louse in the photo can be readily identified as a member of the pigeon louse genus *Columbicola* (Fig. 1c). It may be the cosmopolitan species *Columbicola columbae* (L., 1758), one of the most thoroughly studied of all bird lice (Martin, 1934; Stenram, 1956; Rakshpal, 1959; Eichler et al., 1972; Clayton, 1991; Bush et al., 2006, 2019; Fukatsu et al., 2007; Singh et al., 2010; Crespo and Vickers, 2012; Clayton et al., 2015; Boyd et al., 2017; Villa et al., 2019; Baldwin-Brown et al., 2021). Alternatively, it may be the morphologically similar *Columbicola tschulyschman* Eichler, 1942, which also parasitizes rock pigeons (Price et al., 2003).

Photos of unmounted lice taken with a macro lens or with a camera attached to a dissecting scope, as in figure 3J (reproduced here as Fig. 1a), are not usually sufficient to identify lice to the level of species. However, such photos can often be used to

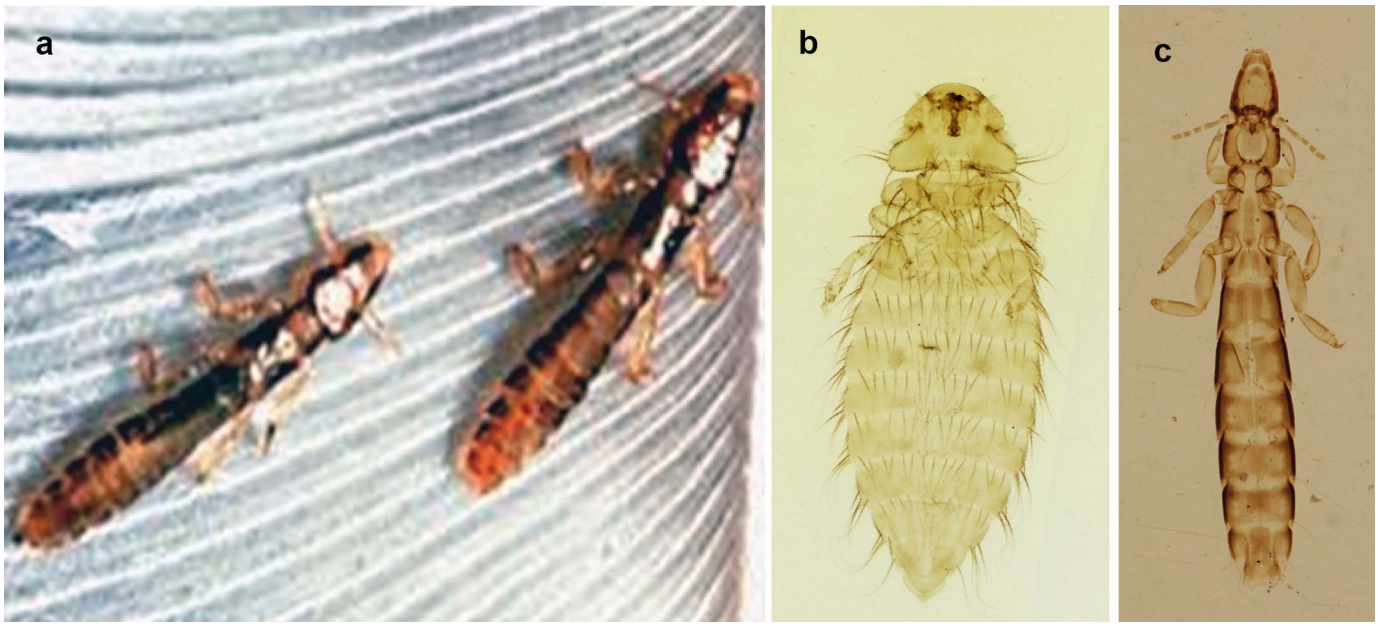

Figure 1. (**a**) Photograph that was misidentified as *Menopon gallinae* by Ali et al. (2020, their fig. 3J), compared to online micrographs of (**b**) *M. gallinae* (NHMUK 010659552) and (**c**) *Columbicola columbae* (NHMUK 010672180) from The Natural History Museum, U.K. (https://data.nhm.ac.uk). *Menopon gallinae* is a parasite of chickens and other galliform birds; *C. columbae* is a parasite of rock pigeons (Columbiformes) (Price et al., 2003). Color version is available online.

identify lice to genus by comparing them to good quality images in the primary or secondary literature. High quality images are also rapidly becoming available in free online repositories (Fig. 1b, c). For example, The Natural History Museum (NHM) in London, U.K., is in the process of digitizing its entire collection. High resolution images of specimens at NHM are freely accessible (see https://data.nhm.ac.uk). Other collections are following suit, with digital access to specimens in more than 1,500 collections worldwide now accessible from a single aggregating website: Global Biodiversity and Information Facility (www.gbif.org). The use of reliable online resources can go a long way toward avoiding specimen misidentification.

Goniodes dissimilis Denny, 1842, the other louse reported by Ali et al. (2020), is a host-specific chicken louse known only from 3 species of *Gallus* (Price et al., 2003). The source of this identification is even more puzzling because *G. dissimilis* appears in Mansur et al. (2019) only as a single entry in a table of lice reported from poultry in Algeria. We speculate that the second louse is a member of one of the following pigeon lice species: *Bonomiella columbae*, *Campanulotes compar*, *Coloceras damicorne*, *Colpocephalum turbinatum*, or *Hohorstiella lata* (Price et al., 2003). Unfortunately, since there is no photograph or other representation of the second louse, there is no way to confirm its identity.

Identification of helminth parasites is more challenging. Improper fixation or mounting can render these parasites difficult, if not impossible, to identify. The photographs of helminths in figure 3 of Ali et al. (2020) are not sufficient to allow confident identification. The cestode and nematode specimens were poorly prepared (e.g., nematodes should not be stained), and the images do not show the important morphological characters needed to identify the specimens. The photos do reveal capsules with single eggs in gravid proglottids, which is a useful taxonomic character. Based on the latest taxonomic work (Jones and Bray, 1994), this feature, together with the shape of the crown of rostellar hooks, suggests that the cestodes are members of either *Cotugnia* Diamare, 1983 or *Paroniella* Fuhrmann, 1920 (formerly classified as a subgenus of *Raillietina* Fuhrmann, 1920). These genera can be differentiated based on the number of sets of genital organs (2 per segment in *Cotugnia* vs. 1 per segment in *Paroniella*), but these traits are not visible in figure 3 of Ali et al. (2020). Members of both genera are common parasites of pigeons (Mlviya and Dutt, 1969; Movsessian, 2003a, 2003b; Zahrani et al., 2012; Biswal et al., 2015).

Of course, misidentifications happen, and errors can never be completely avoided. However, misidentifications in the peer-reviewed literature, such as those by Ali et al. (2020), are increasingly common (e.g., Al-Barwari and Saeed, 2012; Flaiyyh and Kadhim, 2014; Al-Mayali and Kadhim, 2015; Saikia et al., 2017; Abdullah et al., 2018; Arijo et al., 2018; Laku et al., 2018; Ometto-Stolf et al., 2018; Mehmood et al., 2019; Kafutshi et al., 2020). In the case of lice, misidentifications often involve specimens from domesticated chickens, pigeons, ducks, or turkeys (i.e., readily accessible hosts). The specimens are seldom prepared as microscope slide mounts that can be identified to species. Moreover, these papers often cite only literature in regional journals or secondary sources that perpetuate misidentifications (e.g., Soulsby, 1982).

More alarming, perhaps, is the tendency of some authors to blatantly ignore feedback from anonymous peer reviewers. As one example, a recent manuscript with misidentified lice was submitted sequentially to 3 different journals. One of us reviewed the manuscript for each of these journals. Despite providing careful, constructive comments, the comments were largely ignored in each subsequent submission of the manuscript. The paper was eventually published in a fourth journal, more or less

unchanged from the original version of the manuscript. This "damn the torpedoes" approach, which the authors of this article have all experienced as reviewers, is made easier by predatory or semi-predatory journals (Xia et al., 2015; Demir, 2018). In some cases, journal editors have refused to publish corrigenda correcting misidentifications, forcing such corrections to be published in an entirely different journal; see González-Acuña and Palma (2020) for an example. These procedures, and others, are a threat to the integrity of parasitological research.

Misidentification of specimens create error cascades when misidentifications are repeated in later studies (Bortolus, 2008; Wägele et al., 2011). Papers based on such work lead to inaccurate comparisons, misleading mathematical models, and spurious conclusions, occasionally with serious consequences. Identification errors have been responsible for mismanagement of wildlife resources (Shea et al., 2011), ineffective conservation of threatened and endangered species (Beerkircher et al., 2009; Ely et al., 2017), and the misuse of funds meant to help control vectors of human pathogens (Van Bortel et al., 2001).

Consequences of specimen misidentification in molecular studies are equally disruptive. GenBank sequences are sometimes associated with misidentified specimens (Bruns et al., 2008; Collins and Cruickshank, 2013). These misidentifications are promulgated when similar sequences are reported in subsequent studies. Fortunately, misidentifications involving genetic data can be corrected retroactively using matching sequences from properly identified voucher specimens deposited in collections or other repositories at a later date.

In an attempt to rectify the problem of misidentification, previous authors have called for more information to be included in the methods sections of specimen-based papers (Bortolus, 2008; Kholia and Fraser-Jenkins, 2011; Wägele et al., 2011; Vink et al., 2012; Ely et al., 2017; Lutz et al., 2017; Packer et al., 2018; Simon, 2018; Galbreath et al., 2019). At a minimum, authors should state how the specimens were identified, with sufficient detail to allow identification to be verified. We suggest the following guidelines should be used by authors, editors, and reviewers in the biological community to help guard against parasite misidentification:

1. Literature: Rely upon taxonomic publications for specimen identification, such as monographs, original descriptions, identification keys, revisions, or faunal catalogues. If the study material is identified by consulting published literature, list the characters used in the identification of specimens, and cite the reference material. It is never appropriate to simply choose a name from a host-parasite list.
2. Images: Include images of specimens showing morphological characters relevant for identification. Good images will allow specialists to catch and correct mistakes. Images should be in the main text of the publication, or in supplemental online files.
3. Morphological comparisons: If study material is identified through comparisons with previously identified material, such as museum specimens, provide a list of the specimens used and where they are deposited. Although direct comparison with physical specimens is the gold standard, high quality imaging and specimen digitization also make virtual comparisons feasible (see https://data.nhm.ac.uk and gbif.org). Ideally, new specimens should be deposited in collections with long-term storage facilities accessible to researchers, such as natural history museums.
4. Molecular comparisons: If genetic data, such as DNA barcodes, are used for identification, the sequences should be deposited in GenBank (or equivalent), and accession numbers should be provided. This method of identification assumes that genetic data used for identification are associated with correctly identified specimens (Collins and Cruickshank, 2013). When possible, voucher specimens associated with genetic data (preferably hologenophores) should be deposited as in guideline 3.
5. Consultation: Seek assistance from taxonomists and acknowledge their contributions, or include them as co-authors when this is warranted.

In conclusion, we encourage parasitologists to use these guidelines when they write and review papers that depend on specimen-based research. If journals enforce such guidelines, just as many journals now require archiving of original data sets (Whitlock et al., 2010), it will help to protect the integrity of parasitological research.

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