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Morphology is not always useful for diagnosis, and that's ok: Species hypotheses should not be bound to a class of data. Reply to Brown and Gibbons (S Afr J Sci. 2022;118(9/10), Art. #12590)

Significance:

This paper serves as a reply to the Commentary by Brown and Gibbons (S Afr J Sci. 2022;118(9/10), Art. #12590) on our recently published paper on systematics of the moon jellyfish genus *Aurelia* (Lawley et al. PeerJ 2021;9, e11954)). We emphasise that we are not advocating for the routine use of molecular data alone in taxonomic diagnoses, rather that it is a valid approach in cases where, after detailed analyses, morphological features are shown to be unreliable.

We thank Brown and Gibbons[1] for commenting on our work[2], and also the *South African Journal of Science* for providing us open space to debate ideas and points of view that deserve discussion.

Brown and Gibbons[1] are concerned that adopting the approach we recently took in advancing systematics of moon jellyfish in the genus *Aurelia* Lamarck, 1816[3] might lead to 'fleets of autonomous underwater vehicles (AUVs) moving around the world's oceans throwing out new species descriptions on a regular basis using on-board molecular technologies that link to land-based supercomputers by satellite feeds'[1]. While we do agree with the authors' sentiment that AUV-based eDNA monitoring would help build understanding of molecular diversity, we would not want our approach to be seen as justification that new species could or should be created based on such sampling efforts. We do not advocate for such an idea, and indeed our paper concludes by suggesting that more investigation of morphological characters is needed to fully understand diversification in *Aurelia*.

Brown and Gibbons[1] state that we relied on molecular markers alone in our approach tackling the systematics of *Aurelia*, but this is not the case. They omitted our detailed analysis of 40 morphological characters from 173 specimens, either freshly collected or preserved in museum collections, which follows the standard that has been adopted for *Aurelia* for the past 20 years (mostly based on Dawson[4] and used since in most studies that address *Aurelia* morphology[5-7]). Some of the preserved specimens analysed had been previously identified to species level by their collectors, but after our analyses, many of the relevant characters used for those identifications overlapped across distinct localities, where the proposed species were not known to occur. Two such examples are *Aurelia labiata* Chamisso & Eysenhardt, 1821[8] and *Aurelia limbata* Brandt, 1835[9]. Both species were first described around 200 years ago and their morphological diagnoses overall seemed to withstand the test of time: *A. limbata* from the North Pacific with a brown bell margin and highly ramified radial canals[10], and the redescribed *A. labiata* from the northeastern Pacific with a prominent manubrium[11]. Mayer[12] had already reported the prominent manubrium not only in specimens from the Pacific, but also the Indian Ocean, such as in *Aurelia maldivensis* Bigelow, 1904[13]. We also observed this feature in specimens outside of the northeastern Pacific, from Japan, the western coast of Panama, and the Atlantic Ocean off Portugal. None of the genetic sequences obtained from these regions (or nearby) fell within the *A. labiata* clade, providing evidence that the prominent manubrium is likely not a species-specific trait.

Regarding *A. limbata*, Mayer[12] had already considered it a variety of *A. labiata*, which was further suspected by Gershwin[11], who demonstrated that northern morphs of *A. labiata* had more ramified radial canals compared to southern morphs. In our study[2], the highest number of radial canal branching points was observed in specimens from Japan and Arctic Alaska, USA, which coincided with the distribution of two clades in the molecular phylogeny, one considered as *A. limbata* in the northwestern Pacific and the other resurrected as *Aurelia hyalina* Brandt, 1835[9] occurring on the North Pacific and northwestern Atlantic Oceans. Similar to the case of *A. labiata*, this indicated that the brown bell margin and the highly ramified radial canals were also not specific to a single species. We thoroughly reviewed around 200 years of studies, which involved numerous specimens, and they did not reveal any morphological characters that could be used to diagnose these species.

Brown and Gibbons[1] criticise our incorporation of sampling locality into our assessment of species groups. There are indeed shortcomings of using sampling locality as one of the proxies for species grouping, as discussed in our paper[2] (see section 'Molecular analyses, species delimitation and descriptions'). Yet, when considering all the evidence accumulated so far for *Aurelia*, morphology can be an even trickier guide, as illustrated by *A. labiata* and *A. limbata*. To highlight this further, let us consider two more recent examples: the redescriptions[5] of *Aurelia solida* Browne, 1905[14] and *Aurelia coerulea* von Lendenfeld, 1884[15], which Brown and Gibbons[1] emphasise as examples from a taxonomic study that used multiple specimens to incorporate variability. In the case of *A. solida* (type locality Maldives), its designation among Mediterranean specimens was based on the direction of the rhopalium, which was angled up to 90° towards the exumbrella.[5] However, we observed this feature in specimens from locations where *A. solida* has not been reported, from the Atlantic Ocean off Portugal and the southwestern coast of the USA – the latter a region that has been relatively well sampled for the genus in the past 20 years since the first genetic studies for *Aurelia* were published.[16-18] Considering how variable morphology can be within the genus, we hypothesised that this was not a species-specific feature. We maintained only the lack of an endodermal ocellus as a diagnostic feature for *A. solida*, as it tends to fade in preserved specimens and we could not observe it in the analysed samples. However, the recent description of *Aurelia pseudosolida* Garić & Batistić, 2022[19] shows that this species also lacks an endodermal ocellus, and therefore another feature that is not specific to *A. solida*.

In the case of *A. coerulea*, our paper shows that the morphological diagnosis provided in the species' redescription failed, for both continuous and categorical features, as we compared it to laboratory-raised specimens that had been previously identified from molecular data. The diagnosis should be applicable to the taxa (as indicated by the International Commission on Zoological Nomenclature[20]; see code's glossary for 'diagnosis'), no matter if cultured in the laboratory or wild-caught. The redescription of *A. coerulea* and *A. solida* used multiple medusae (as did studies of *A. labiata* and *A. limbata* over the course of 200 years), yet their morphological diagnoses still failed (for more details and further examples, see the remarks for each species in the 'Systematic account' section of our paper[2]). Indeed, our study[2] details our thorough investigation of morphological characters for their potential to diagnose species. It is only in light of such a comprehensive review that one might consider the exclusive application of molecular characters for species diagnoses within a group.

Brown and Gibbons[1] fear that our approach could lead to the rise of parallel, non-overlapping taxonomies, with names delimited via molecular analysis and names based on descriptions that render the species unrecognisable. Parallel (or even pseudo-) taxonomies already exist. Since the turn of the century, a swarm of putative species has arisen from lineages identified through molecular species delimitations, especially for cryptic taxa, which in many cases appear with informal naming or numbering schemes. These informal names end up used in the literature and treated as species hypotheses, already seeding confusion before the species are formally described. *Aurelia* sp. 1 for example, introduced based on molecular data[16] and later formally recognised as *A. coerulea*[5], has appeared in 26 articles, even after its formal recognition and redescription (based on a Web of Science search of "*Aurelia* sp. 1" in All Fields)[21], which includes the paper that reported an *Aurelia* genome[22]. This issue is not limited to *Aurelia*. In fact, within the NCBI's taxonomy database, the proportion of 'sp.' species among the classes of Medusozoa range from 25.8% to 58.9%, with the highest value in Scyphozoa[23], which further emphasises the gap between species delimitation and description. The question, and of course the controversy, remains: how do we bridge this gap?

Morphology has always been essential for evolutionary studies, including taxonomy, and it should always be incorporated as well as possible. However, if proven unreliable for diagnosis, taxonomists should not be obligated to stick to it (other examples of diagnoses based on molecular data include tapeworms[24], slugs[25] and amphipods[26]). The concern should be to provide testable hypotheses about the structure of biodiversity, which can be corroborated or refuted when new data become available.[24,27] Our study was an earnest attempt to reconcile species delimitation with descriptions, and offer a basis for future studies, even if in some cases based on a single or few individuals. This is not uncommon practice within Medusozoa, especially considering the patchy distributions and rarity of many species. One recent example is *A. pseudosolida*, which was described based on a single specimen detected within a bloom of *A. solida* in the Adriatic Sea.[19] We are not questioning the validity of that study, merely illustrating that this is still done. Indeed, Brown and Gibbons[1] point out that it is reasonable to describe species based on single specimens if their appearance is obviously different. The same argument should apply to molecularly distinctive taxa when a group is well characterised genetically (especially considering a cryptic taxon such as *Aurelia*).

As mentioned by Packer et al.[28]: '…it is perhaps ironic that new species are readily described on the basis of subtle morphological variation, yet there is a general reluctance to describe species on the basis of genetic evidence alone…'. Furthermore, 'when 10% of taxonomic diversity has been discovered in 250 years, no technological breakthrough is likely to make it possible for us to describe the remaining 90% in a shorter time period.'[28]

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