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Philippe Borsa. Comments on “Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity” (Weigmann, 2016) . Journal of Fish Biology, 2017, 10.1111/jfb.13235 . ird-01532870

HAL Id: ird-01532870

<https://ird.hal.science/ird-01532870>

Submitted on 3 Jun 2017

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To be cited as: Borsa P (2017) Comments on “Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity” (Weigmann, 2016). Journal of Fish Biology 90, 1170-1175.

Comments on “Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity” (Weigmann, 2016)

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S. Weigmann recently synonymized the fine-spotted leopard whipray *Himantura tutul* (Myliobatoidei: Dasyatidae) with the darkspotted whipray *Himantura uarnak*, and the New Caledonian maskray *Neotrygon trigonoides* (Myliobatoidei: Dasyatidae) with the blue-spotted maskray *Neotrygon kublii*. However, *Himantura tutul* is genetically distinct, reproductively isolated, and partly distinct morphologically from the leopard whipray *Himantura leoparda* with which it was previously confused, and both are morphologically and genetically distinct from *H. uarnak*. Likewise, *N. trigonoides* is a genetically and morphologically distinct species from the Coral Sea and possibly the adjacent Vanuatu and Fiji archipelagoes.

Key words: *Himantura tutul*; *Neotrygon trigonoides*; synonymy; nucleotide sequence; colour pattern

Taxonomy has traditionally relied on morphology to distinguish species. There is no reason, however, to dismiss genetic evidence in the description or diagnosis of species, or to claim genetic evidence is acceptable only when it supports morphological evidence (Vogler & Monaghan, 2007; Cook *et al.*, 2010; Jörger & Schrödl, 2013). Within the past few decades, the generalized use of molecular markers in population genetics and in phylogeny has led to delineating cryptic species that previous morphological studies had failed to detect. Several such examples do exist in chondrichthyans (Weigmann, 2016). As a consequence, the input from genetic markers has led to a remarkable upsurge in species descriptions, re-descriptions, synonymizations and resurrections (White & Last, 2012; Weigmann, 2016; and references therein). Weigmann (2016) provides an annotated checklist of the chondrichthyan fishes of the world, which constitutes a useful update on the nomenclature, taxonomy and distribution of chondrichthyans. This checklist, however, dismissed several recently-published taxonomic reports and took nomenclatural decisions that are challenged here.

Weigmann (2016: 138) synonymized the fine-spotted leopard whipray *Himantura tutul* Borsa, Durand, Shen, Arlyza, Solihin & Berrebi 2013 with the darkspotted whipray *Himantura uarnak* (Gmelin (ex Forsskål) 1789) by writing: “*Himantura tutul*... is a junior synonym of *H. uarnak* due to strong morphological resemblance. This is also confirmed by molecular data (Naylor, 2015, pers. comm.)”. Forsskål (1775) originally described *Raja uarnak* after *Raja sephen* Forsskål 1775, in the following terms: “*Raja. Arab. Uárnak, similis descriptae, sed tota maculata, spina una vel duplici in cauda, quae apterygia*”. By using the latin adjective *maculata*, it is sensible to assume that P. Forsskål referred to solid spots. Had he wished to describe the leopard-like spots characteristic of the leopard whipray *Himantura leoparda* Manjaji-Matsumoto & Last 2008 or the finer leopard-like spots of *H. tutul*, he probably would have instead used the latin adjective *ocellata*. *Himantura uarnak* is understood by taxonomists as the whipray species having densely and regularly spaced, solid, round or oblong dark spots all over the surface of the dorsal side (Fig. 1) (Rüppell, 1835; Duméril, 1865; Randall, 1995; Last & Compagno, 1999; Manjaji, 2004; Arlyza *et al.*, 2013b). A comparison of the pigmentation patterns of *H. uarnak* with those of its three relatives *H. leoparda*, *H. tutul* and *H. undulata* Bleeker 1852 is available from Arlyza *et al.* (2013b: Graphical abstract). Whiprays with the typical pigmentation patterns of *H. uarnak* do occur in the Red Sea, the type locality of the species (Rüppell, 1835; Mikalauskas, 2010; S. Bogorodsky, pers. comm.). Unfortunately, no nucleotide sequence of *H. uarnak* from the Red Sea is yet available. This should be a priority for taxonomic investigation, as emphasized by Naylor *et al.* (2012). A leopard-like spotted specimen with the robe typical of *H. tutul* has been recently captured in the Eastern Mediterranean (Ali *et al.*, 2010). If confirmed by molecular markers, this observation would imply that *H. tutul*, which is typically an Indo-West Pacific species, should also occur in the Red Sea. Mitochondrial phylogenetic trees of the *H. uarnak* species complex produced using different portions of the mitochondrial DNA and different samples of individuals are summarized in Fig. 2. The tree represented on Fig. 2a is based on the partial cytochrome *b* gene sequences (260 bp) concatenated with the partial 16S rDNA sequences (ca. 580 bp) provided by Manjaji (2004). In this phylogeny, the ‘*H. leoparda*’ haplogroup is paraphyletic with *H. undulata* and *H. uarnak*. Based on its cytochrome *b* gene sequence, specimen CSIRO H5478.01 (paratype of *H. leoparda*) belongs to clade IV of Arlyza *et al.* (2013b), i.e. *H. leoparda*. The rest of the haplogroup (dotted rectangle on Fig. 2a) concerns five specimens all initially

determined as *H. leoparda* (then *Himantura* sp. A; Manjaji, 2004) including *H. leoparda* paratype CSIRO H5284.05 but which clustered with clade *I* of Arlyza *et al.* (2013b), i.e. *H. tutul*. In other words, five out of six of the specimens identified as *H. leoparda* on the basis of morphology (Manjaji, 2004) actually belong to *H. tutul* according to their mitochondrial type. The complete mitochondrial DNA of a specimen of *H. leoparda* from the South China Sea (Shen *et al.*, 2016) effectively confirms that the *H. leoparda* clade of Fig. 2c is identical to that of Fig. 2b. Manjaji-Matsumoto & Last (2008: 298) also distinguished *H. leoparda* (hence, *H. tutul*, then confused with it) from *H. uarnak* and *H. undulata* by the arrangement of the midscapular denticles. Pigmentation patterns in adults may be useful to tentatively link the four other clades of Naylor *et al.* (2012) (Fig. 2b) with the three remaining clades of Borsa *et al.* (2013b) (Fig. 2c). This information was retrieved from the Elasmobranch Specimens section of the Global Cestode Database hosted by the University of Connecticut (<http://tapewormdb.uconn.edu/>). Clade *uarnak* 1 of Naylor *et al.* (2012) includes presumed adult specimens (> 800 mm disk width) BO-309, BOD-73 and KA-411 that mostly possess regularly spaced, solid, round or, in some specimens, elongate but non-intricate dark spots over the dorsal surface. This corresponds to the typical pigmentation pattern of *H. uarnak*, making the latter a potential candidate for *uarnak* 1. Clade *uarnak* 2, the sister-clade to *uarnak* 1 includes presumed adult specimens with numerous dark points (SO-4), or small dark spots (SO-16) that appear to differ from typical *H. uarnak* patterns. Naylor *et al.* (2012) sampled *uarnak* 2 haplotypes exclusively from northern Australia, which makes this clade the potential homolog of Borsa *et al.*'s (2013b) 'NWA' sub-clade of *H. uarnak* (Fig. 2c). Whether *uarnak* 2 is a geographical variant of *H. uarnak*, or another biological species in the *H. uarnak* species complex remains to be investigated. The pigmentation patterns of one of the large-sized specimens of Naylor *et al.*'s (2012) clade *uarnak* 3 (BO-422) are typical of *H. tutul*, making *H. tutul* a likely candidate for *uarnak* 3. Naylor *et al.*'s (2012) clade *uarnak* 4 was represented by the haplotype of a single individual with no information on colour patterns. Further research is necessary to verify whether clade *uarnak* 4 characterizes yet another species in the *H. uarnak* species complex. To help ascertain which of Naylor *et al.*'s (2012) clades *uarnak* 1-4 (Fig. 2b) is *H. tutul* and which one is *H. uarnak* would require the sequencing of an additional number of specimens at both the *ND2* and *CO1* or cytochrome *b* loci. However, based on pigmentation patterns, *uarnak* 1 might represent *H. uarnak* while *uarnak* 3 is likely *H. tutul*.

In summary, *H. tutul* was previously confused with *H. leoparda* (Manjaji, 2004; Manjaji-Matsumoto & Last, 2008) from which it is yet genetically distinct, reproductively isolated, and partly distinct morphologically (Arlyza *et al.*, 2013b; Borsa *et al.*, 2013b). The mitochondrial haplotypes of specimens of *H. uarnak* sampled from the Indo-Malay region and from northwestern Australia cluster as a clade distinct from *H. tutul* (Figs. 2a & c). Thus, there is no evidence to place *H. tutul* as a junior synonym of *H. uarnak*.

Weigmann (2016: 139) also dismissed Borsa *et al.*'s (2013a) resurrection of the New Caledonian maskray *Neotrygon trigonoides* (Castelnau 1873) by stating "... Borsa *et al.* (2013) resurrected *Raya trigonoides*... as a valid species for specimens of *N. cf. kuhlii* from off New Caledonia. The resurrection is, however, problematic as neither a detailed description nor morphometrics were given. Furthermore, specimens that were morphologically very similar to '*N. trigonoides*' were caught in the Gulf of Oman in 2013 (Weigmann, unpubl. data), although the species is confined to New Caledonia according to Borsa *et al.* (2013)". Castelnau's (1873) *N. trigonoides* was declared a junior synonym of the blue-spotted maskray

Neotrygon kuhlii (Müller & Henle 1841) by Last & White (2008), but no valid explanation was given for this nomenclatural act. Borsa *et al.* (2013a) found that specimens identified as *N. kuhlii* from the Coral Sea differed from *N. kuhlii* as depicted by Müller & Henle (1841: pl. 51) by the possession of a dark blotch on each shoulder and by the presence of dark spots (> 1% disk width) on the dorsal side. All specimens with a scapular blotch that were genetically analyzed formed a monophyletic haplogroup sister to the haplogroup that comprised all typical *N. kuhlii* specimens, i.e., possessing blue ocellated spots and dark speckles, but no scapular blotch and no dark spots (Borsa *et al.*, 2013a). No specimen from Oman was then available for genetic analysis. Specimens identified as *N. kuhlii* from the Coral Sea were sufficiently distinct, both morphologically and genetically from *N. kuhlii* from the other side of the Torres Strait to be considered as a separate species, for which Borsa *et al.* (2013a) resurrected the name *N. trigonoides*. Based on genetic data alone (Borsa *et al.*, 2013a; Puckridge *et al.*, 2013), the current known distribution of *N. trigonoides* includes eastern Australia from Lizard Island to northeastern New South Wales, and New Caledonia. Based on pigmentation patterns, the species may also be present in the adjacent Santa Cruz, Vanuatu, and Fiji archipelagoes. Extensive genetic surveys (Arlyza *et al.*, 2013a; Borsa *et al.*, 2013a; Puckridge *et al.*, 2013) failed to detect *N. trigonoides* north west of the Torres Strait, or throughout the Coral Triangle, or west of it. Weigmann's (2016) report of a specimen from Oman that he claimed to be “*morphologically very similar*” to *N. trigonoides* does not necessarily imply that *N. trigonoides* is also present in Oman. Neither does this constitute evidence that *N. trigonoides* might be synonymous to *N. kuhlii*. Sequencing the mitochondrial DNA of Weigmann's specimen from Oman would be helpful to verify whether it is related to *N. trigonoides*. The main point here is that, so far, there is no reason to once again synonymize *N. trigonoides* with *N. kuhlii*.

I am grateful to P. Berrebi, J.-D. Durand, N. Hubert, K.-N. Shen and R.D. Ward for valuable discussions, and to I.J. Harrison and an anonymous reviewer for thoughtful comments and suggestions. E. Rüppell's *Fische des rothen Meeres* and A. Duméril's *Ichtyologie générale* were consulted online from the Biodiversity Heritage Library website (<http://www.biodiversitylibrary.org/>). Authors' manuscript versions and supplementary information to the papers that describe *H. tutul* and resurrect *N. trigonoides* are available from the open-access HAL-IRD website (<http://www.hal.ird.fr/>).

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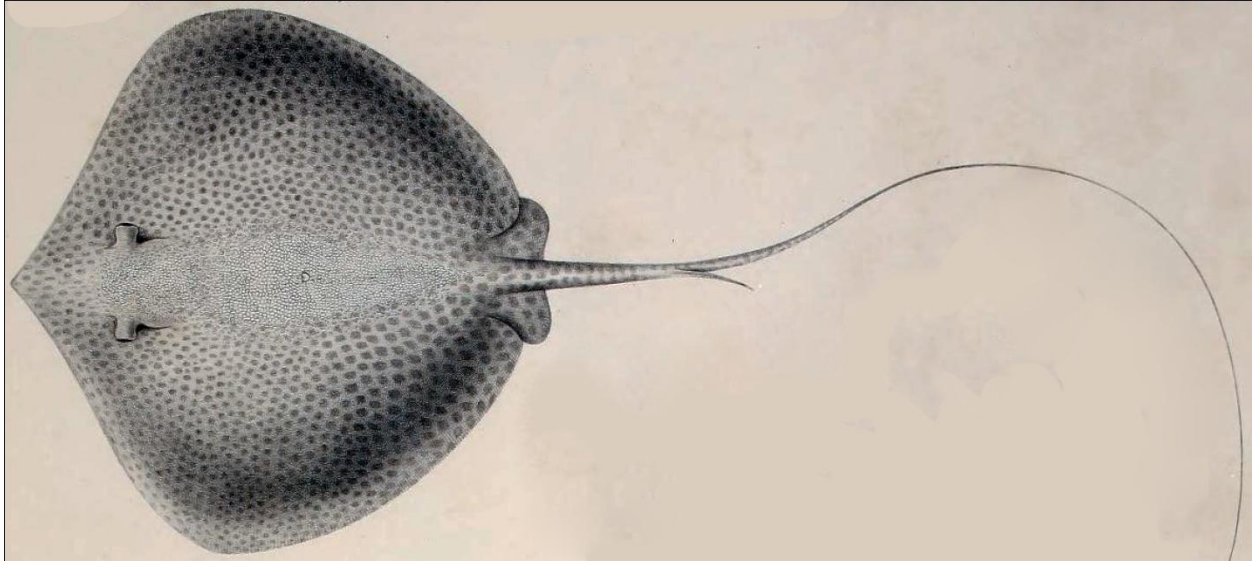


FIG. 1. *Himantura uarnak* from the Red Sea as represented by Rüppell (1835: plate 19); edited for clarity.

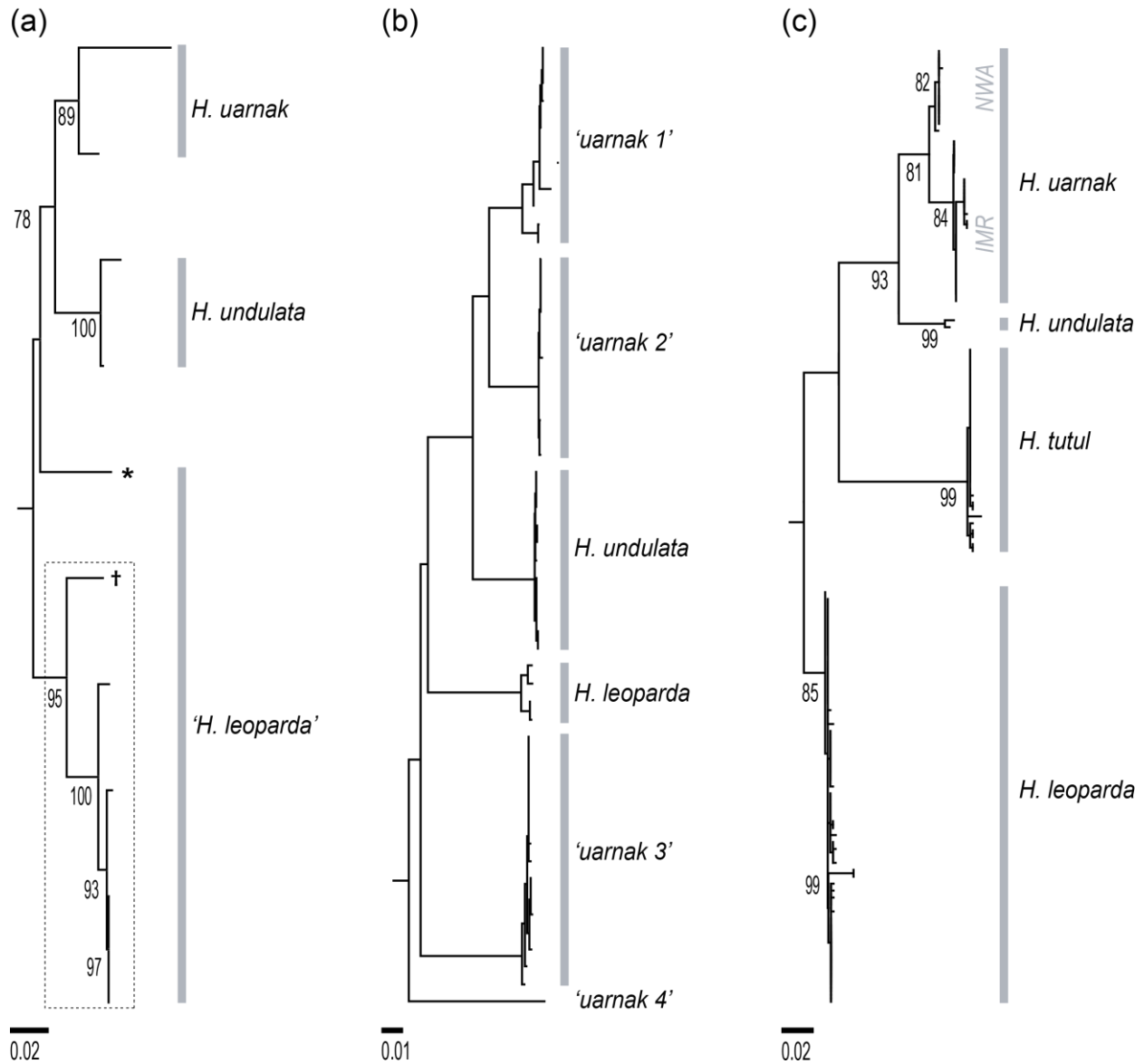


FIG. 2. Mitochondrial phylogeny of the *Himantura uarnak* species complex. (a) Neighbor-Joining tree based on concatenated nucleotide sequences of cytochrome *b* gene and 16S rDNA fragments (Manjaji, 2004) [MEGA5 (Tamura *et al.*, 2011); K2P model; pairwise deletion; tree rooted by *H. signifier*]; asterisk (*) designates specimen CSIRO H5478.01, one of the paratypes of *H. leoparda*; cross (†) designates specimen CSIRO H5284.05, another paratype of *H. leoparda*; dotted line delineates the sub-clade subsequently identified as *H. tutul* (Arlyza *et al.*, 2013b; Borsa *et al.*, 2013b); numbers at nodes are bootstrap scores (when >50%). (b) ND2 gene-based phylogeny of Naylor *et al.* (2012). (c) Phylogeny of Borsa *et al.* (2013b) based on nucleotide sequences of partial CO1 gene; homology of clades *H. uarnak* and *H. undulata* with those of Fig. 2a was established by simultaneously sequencing a proportion of individuals at the cytochrome *b* locus (Arlyza *et al.*, 2013b). IMR Indo-Malay region; NWA northwestern Australia.