Computer-aided photo-identification of a rare stingray, *Megatrygon microps*.

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ABSTRACT
We have defined protocol for photo-identification to identify individual *Megatrygon microps*. 104 identification photographs were taken between 2005 and 2019. Spot patterns on the dorsal surface were used to identify individuals. Unique scarring on 8 re-observed *M. microps* provided an independent confirmation of pattern stability of up to 761 days. Previously, studies have lacked statistical testing to validate photo-identification approach. The I^{3}S photo-matching software was proven to successfully match images, identifying 69 individuals. A photo-matching software facilitates an open-source platform for identifying individual *M. microps*, allowing for better population assessments.

KEYWORDS
Megatrygon microps, stingray, photo-identification, I^{3}S

MAIN TEXT
Mark-recapture techniques to monitor individual animals have been used extensively in studies of animal ecology. The ability to individually identify animals in long-term studies makes it possible to document individuals' age-related changes in maturity, behaviour and survivorship, and provides information on population size and demographics (Clutton-Brock and Sheldon, 2010). It is possible to identify individual animals by variations in their natural marks (e.g. skin pigmentation patterns, scars, fin shape) by comparing photographs of an individual taken at different times (Frisch and Hobbs, 2007). Unfortunately, many studies lack independent means of verifying identification, so animals with similar skin pigmentation patterns are assumed to be of the same individual without evidence. Photo-matching software has been developed to more efficiently match images of unique individuals whilst

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reducing the risk of human-error in photo matching (Matthé *et al.*, 2017). Photo-matching software makes it possible to facilitate an open-source platform for identifying individuals globally. Providing that sufficient statistical testing validates the matching efficacy of the photo-matching software, this approach is has been shown to be particularly useful for a patchily distributed and widespread species, where animals moving between study areas may be detected using such an identification database (e.g. https://www.whaleshark.org; Arzoumanian *et al.*, 2005).

Megatrygon microps is a large dasyatid species, reaching widths up to 222 cm (Garman, 1913). It is a patchily distributed stingray found in tropical and subtropical seas inhabiting estuarine, river mouth and coastal areas. The few records of this species span the western Indian ocean from Mozambique (Pierce, White and Marshall, 2008) to Townsville in the Great Barrier Reef (Last and Stevens, 2009). Fisheries records include India and Bangladesh (Kapoor *et al*. 2002) including within the river Ganges (Ishihara *et al*. 1998), throughout areas of southeast Asia (Mohsin and Ambak, 1996, White *et al*. 2006) and northern Australia (Last and Stevens, 2009). The World Conservation Union Red List (IUCN) lists *M. microps* as DATA DEFICIENT (Fahmi *et al.*, 2016), calling for more research into the life history, ecology and population trends of the species. Fishing activities occur throughout the known range of *M. microps* (van der Elst and Everett, 2015) and, given the evidence available in the literature, it would seem likely that *M. microps* is vulnerable to decline (Last and Stevens, 2009).

The aims of our study were to establish whether individual *M. microps* could be reliably identified using natural spot patterns and to assess the discriminatory power of a freely available software for pattern-matching. Establishing whether *M. microps* can be individually identified will allow for a large-scale photo-ID programme for this species, and for a better understanding of its population.

5,525 recreational dives were undertaken between 2005 and 2019 over 19 discrete dive sites spanning 30km of coastline in the Inhambane region of southern Mozambique. All dive sites were at depths of 20-30 m and consisted of reefs with a mixture of rocky and sandy benthic substratum. Each sighting of *M. microps* was opportunistically photographed (i.e. when *M. microps* passed near to divers), without chasing the animals. The ideal images were taken perpendicular to the dorsal surface, when both pectoral fin tips reached the flattest possible point (<5° flex) to minimise spot distortion.

Photographs were taken under varying light quality, underwater visibility and camera angles(figure 1). Natural markings on *M. microps* show a highly varied series of large white spot patterns running in parallel along the outer edges of the dorsal surface of the pectoral fin. All spot markings were white/cream against a dark brown/beige dorsal surface of the stingray, creating adequate contrast for identification. Natural markings near to the margins of the pectoral fins were sometimes obscured by white scarring (figure 1a-e), shark bite wounds (figure 1f) and a covering of white/cream mucus and (figure 1g). The pectoral fins are used for propulsion in an oscillatory motion (Pierce, White and Marshall, 2008). This causes distortion in the spot patterning and was the most common issue with obtaining the ideal image, as the animals were always seen in motion.

Prior to computer-aided matching, some images were corrected for fisheye lens distortion and colour corrected using the Capture One Express Sony 12.0© photo editing software. The best photo of each sighting was selected according to criteria describing both picture and mark quality (Urian *et al.*, 2015);

Good picture quality	1. Photograph is focussed on the animal 2. Resolution of photograph is good enough to distinguish markings 3. Fin tips have only slight (approx. <5°) or no flex 4. >90% of dorsal surface is visible (excluding tail) and not obstructed by poor lighting (glare), diver, fish or sediment 5. Camera angle is as close to perpendicular to the dorsal surface of the smalleye as is reasonable
Good marking quality	1. *Megatrygon microps* possesses numerous and clearly defined spots, either clustered or distributed across the dorsal surface

Poor-quality photographs were not used in the present analysis to minimise the risk of incorrect re-identification. Of 104 photographed sightings, 96 were deemed good-quality images suitable for identification.

To confirm the spot stability of natural markings of *M. microps*, the main database of 96 images was compared by eye on a split computer screen in an iterative pairwise comparison process by J. K.. In addition, K. R-A. and N. C. independently compared a subset of the database each performing 900 comparisons in a randomised blind trial. The database subset contained pairs of images which were 'known to be the same individual' and pairs of images which were 'known to be different individuals' and 'unknown' matches. The criteria for such image pairings are outlined below. No disparity in identifications by the three independent researchers were discovered.

Paired images 'known to be the same individual'	1. The paired images were taken consecutively, during the same encounter and never out of view of the photographer. 2. The paired images are marked with identical scarring or deformities.
Paired images 'known to be of different individuals'	1. The pair of images is of two individuals seen simultaneously, both in view of the observer. 2. The paired images are marked with different scars or deformities.

Paired images ‘unknown’ to be of the same or different individuals	1. The pair of images were taken during different encounters and included no identical scarring or deformities. 2. The pair of images were taken during the same encounter, but *Megatrygon microps* left the photographers’ field of view.

Sixty-nine individuals were identified among the 96 good-quality photographs. This main database of images contains twelve scarred/deformed individuals, two of which were re-photographed. These were one male and one female, both of which exhibited a unique and recognizable white scar pattern along the trailing edge of the pectoral fin, a result of a historical injury (figure 1a-e). The male individual was photographed twice during its first sighting and again 761 days later, where the scarring and spot pattern remained stable (figure 1a-c). Of the 69 individual stingrays in the main database of images, eight have been re-photographed during different sightings. Seven stingrays were re-photographed once, and one was re-photographed twice. Re-photograph intervals in these eight cases ranged from 17 to 1025 days (additional information in supporting information). A further eighteen were first sighted and subsequently re-photographed seconds later during their original sighting. In all cases, spot patterns were found to have remained static during these periods.

Within the I^3S photo-matching software, a fingerprint file for each image was constructed as described in the I^3S manual (Hartog and Reijns, 2014). Three reference points over the dorsal region were set at the point of the rostrum and the left and right pectoral fin insertion (figure 1h). Although they do not incorporate the entire region of interest for identification, these reference points are distinguishable in every image and their location is less susceptible to flexion. Spots and spot clusters were manually identified following the protocol described in the I^3S manual and were the same for each reference point trial. Spots used for identification were ≥1cm diameter on the I^3S software interface to prioritise the selection of the most visible spots. No more than 30 spots/spot clusters were defined in each fingerprint (figure 1g). A match score was calculated to represent how closely matched two fingerprints were. The lower the match score, the closer the spots matched between the two fingerprints. The match score ranks each stingray image in the database as to how close their matching spots are.

A Receiver Operating Characteristic (ROC) curve was used to evaluate the efficacy of I^3S software to identify matching images of *M. microps* produced with ROCR package (Sing *et al.*, 2005) in R (version 3.4.4). A 31-image subset of the main database of images was individually compared with the remaining 65 images in the main database in the I^3S software (Hoffmann *et al.*, 2010). A total of 49 paired image match scores were recorded in categories of ‘known to be the same individual’ or ‘known to be different individuals’ or ‘unknown’. Using the ROC curve, we assessed whether a match score taken from the ‘known to be the same individual’ category is likely to have a value lower than a match score from the ‘known to be different individuals’ category (figure 2). An area under the curve (AUC) value was calculated by plotting sensitivity against 1-specificity at all possible threshold probabilities for a positive prediction (Hanley and McNeil, 1982), which ranges from zero to one. The AUC value was interpreted as the probability that a match score is less in paired images of 'known to be the same individual' than with 'known to be different individuals'. The closer the AUC value is to one, the better the predictive power of the I^{3}S software at finding matching individuals. An AUC value of 0.5 would indicate that there was no difference between the efficacy of the I^{3}S software to correctly match individuals and random chance that two correctly matching images from the main database were paired. The AUC value was 0.8027 ($P < 0.001$).

Some criticism has been raised regarding the use of AUC as a measure of performance, especially if observations (in this case, quality of images) are unreliable (Lobo *et al.* 2008). We feel that the use of AUC is reliable in this study, due to the employment of specific 'good-quality image' guidelines. The AUC score favourably demonstrates that the I^{3}S-generated match score of two images of the same individual will be below the match score threshold.

The optimal match score cut-off value is useful to prioritise likely matched individuals. This was calculated by a dot diagram, where a horizontal line represents the optimal match score. A violin plot was overlaid to show the kernel probability density of the data at different values (figure 3). The optimal threshold match score for discriminating between these classes is estimated to be 10.06, with associated sensitivity and specificity rates at 78.3 and 73.1 respectively. This value can be used as a benchmark for future image matching criteria. The mean match score was 7.48 ±3.81 for all re-sighted individuals.

Using the 'Elaborate Evaluation' tool in the I^{3}S software for the main database of 96 images, using 1 reference image per stingray, gives 95.26% probability that when comparing a new image of an individual that is already in the database, the correctly matched individual was in the top 5 search results.

Two primary considerations must be addressed when implementing photo-ID on this species with the current method; (1) Good-quality photographs are easier to attain in favourable underwater conditions. Strong current and poor visibility negatively affected both the ability to photograph a mobile animal and diminished the quality of the photograph. (2) Individuals showing avoidance behaviours, such as banking, changing direction and leaving the area, were less likely to be photographed.

Most stingrays (88.5%) were not re-photographed in this study. Fishing and predation pressures could be contributing to the lack of re-sightings (van der Elst and Everett, 2015). It is also known issue with mark-recapture studies that "trap shyness" can occur, reducing the rate of recaptures and artificially increasing the apparent size of the population. It is also possible that there is a pre-existing avoidance behaviour to divers (Lindfield *et al.*, 2014). Avoidance behaviours that were observed in this study, such as banking, change of direction and leaving the area (pers. obs. J.K.), were assumed to have been induced by the presence of divers. Research is needed to fully understand diver/stingray interactions.

More information is needed to establish if spot patterns remain stable on *M. microps* from birth. Sightings of neonates and juveniles has not yet been documented *in situ*, however spot patterns have been observed in the advanced foetus (Nair and Soundararajan, 1976). If the spot pattern remains stable from juvenile through to sexual maturity, methods such photo-ID would better inform conservation efforts, with information such as movement and behaviour through these vital years of development.

The successful application of a photo-matching software for *M. microps* can be considered a superior alternative to physically capturing the stingray or matching photographs by eye alone. A photo-matching software makes it possible to facilitate a platform unto which many contributors, globally and over a long time period, may maximise survey effort and essentially providing evidence-based decisions on the conservation status of this species.

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SUPPORTING INFORMATION

1. "Match scores of re-photographed individuals"

CONTRIBUTIONS

J. K. conceived and designed the method, collected and analysed data and prepared the manuscript. K. R-A conceived and designed the method, collected and analysed data and edited the manuscript. N. B. analysed the data and edited the manuscript. N. C. analysed the data. D. B. designed the method and edited the manuscript. D. M. edited the manuscript.

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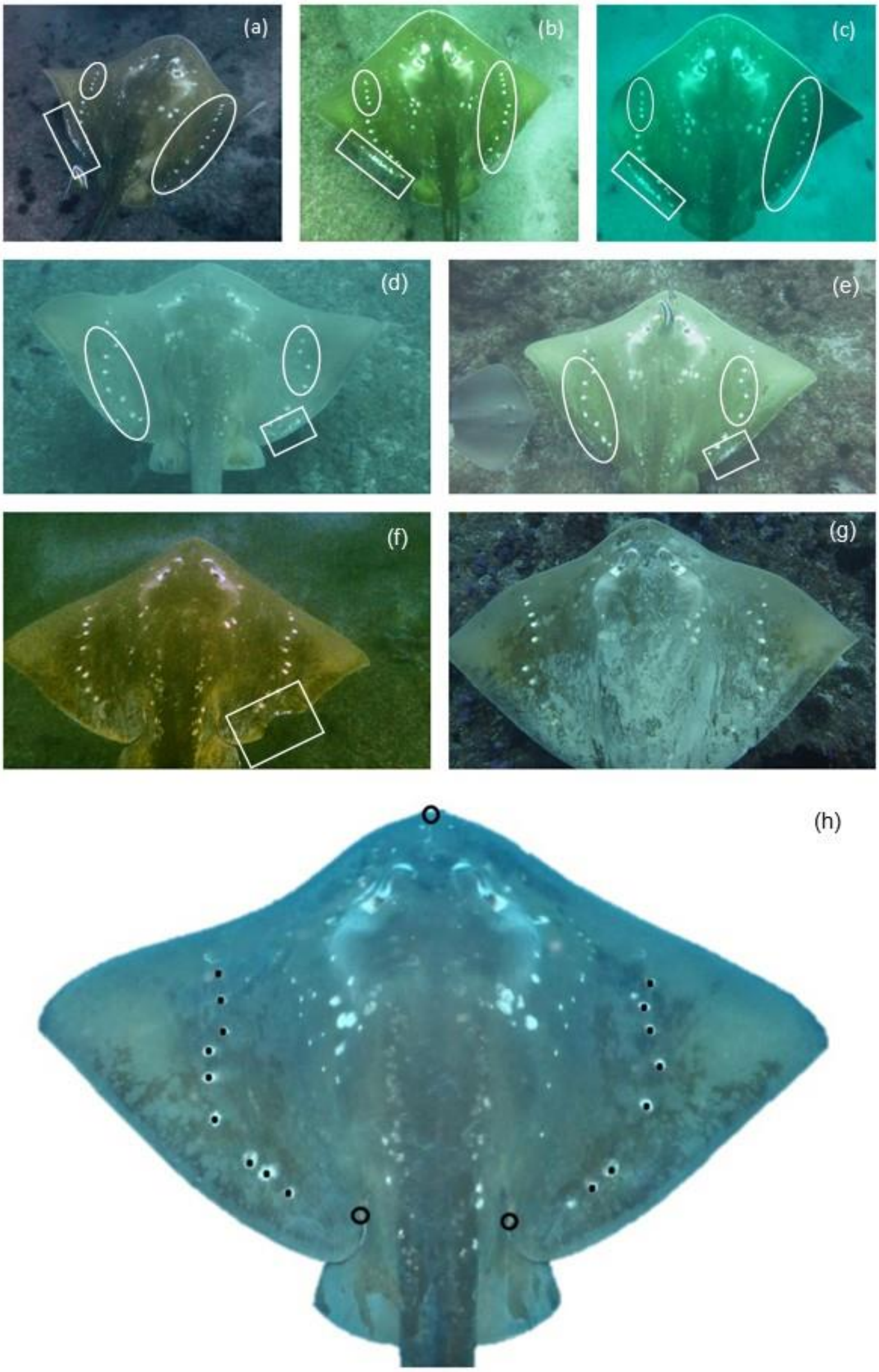
FIGURES

Figure 1. Examples of unchanged natural markings, bite marks, mucus and gender identification of *Megatrygon microps*. Notable spot pattern matches denoted by white ellipses and white rectangles indicates scarring. Individual 120713Giants2 with stable large white scar markings sighted (a) 13.07.2012, (b) during the same encounter a few seconds later and (c) re-sighted 12.8.2014 (761 days later); Individual 130911Outback1 with stable small scar markings sighted (d) 11.9.2013 and (e) 11.5.2015 (608 days later). (f) Bite mark on individual 180818Manta1; (g) Mucus coverage on individual 151112Office1; (h) The three reference points (O) and all markings annotated (•)for *Megatrygon microps* in the I^3S software interface. Also note the varied light, underwater visibility and camera angles.

Figure 2. Receiver operating characteristic (ROC) curve of I^3S match scores indicating the accuracy of discrimination. As the line tends towards 1 (the top left corner of the graph) the more optimal the sensitivity and 1-sensitivty scores become.

Figure 3. Dot diagram with violin overlay of known to be the same and known to be different *Megatrygon microps* image match scores. Horizontal line indicates an estimated optimum I^3S match score cut off value (10.06). Red dots indicate the mean match score value for each category.


(a)
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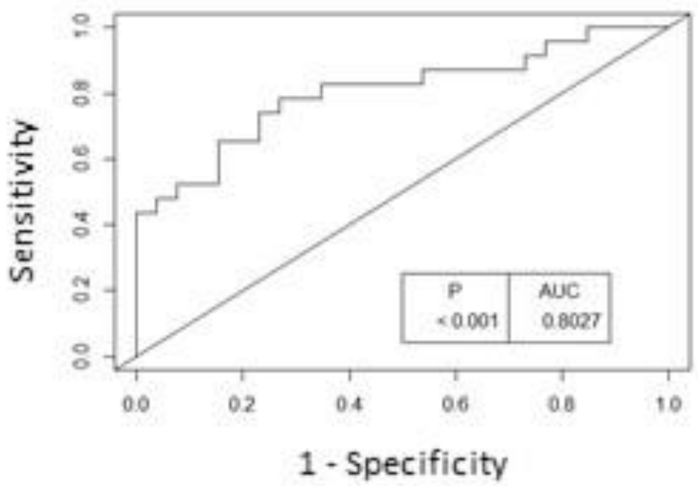
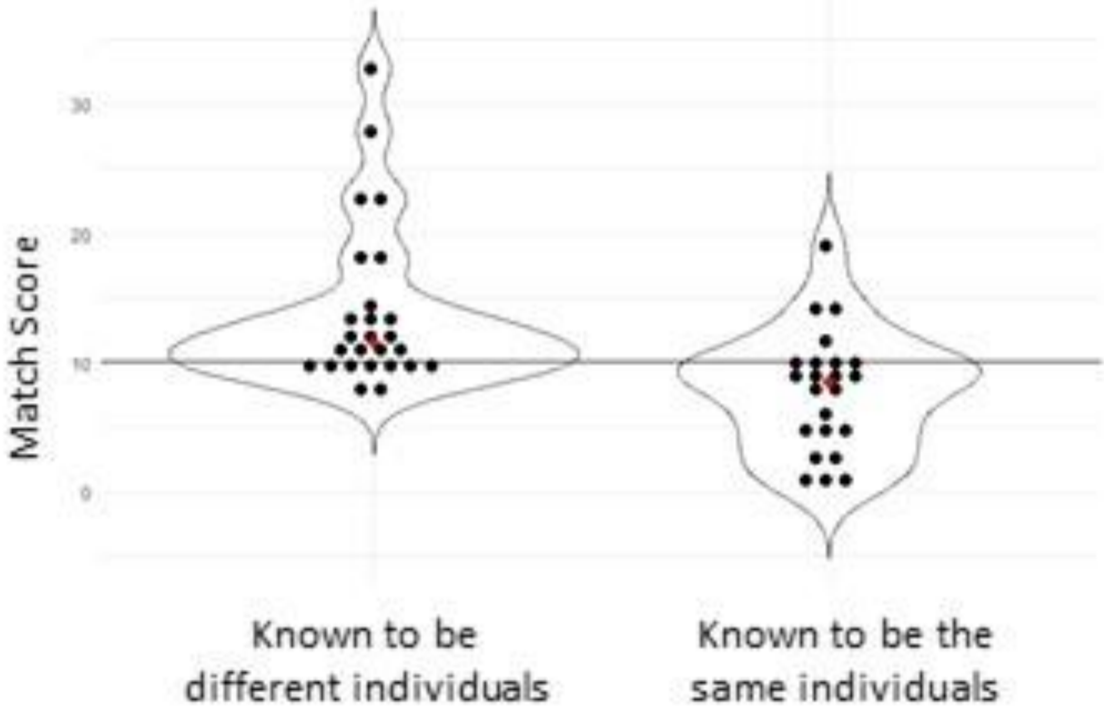

Sensitivity
1 - Specificity
P
< 0.001
AUC
0.8027


Match Score
Known to be different individuals
Known to be the same individuals