

Determining natal origin for improved management of Atlantic bluefin tuna

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Effective sustainable management of marine fisheries requires that assessed management units (that is, fish stocks) correspond to biological populations. This issue has long been discussed in the context of Atlantic bluefin tuna (ABFT, *Thunnus thynnus*) management, which currently considers two unmixed stocks but does not take into account how individuals born in each of the two main spawning grounds (Gulf of Mexico and Mediterranean Sea) mix in feeding aggregations throughout the Atlantic Ocean. Using thousands of genome-wide molecular markers obtained from larvae and young of the year collected at the species' main spawning grounds, we provide what is, to the best of our knowledge, the first direct genetic evidence for "natal homing" in ABFT. This has facilitated the development of an accurate, cost-effective, and non-invasive tool for tracing the genetic origin of ABFT that allows for the assignment of catches to their population of origin, which is crucial for ensuring that ABFT management is based on biologically meaningful stock units rather than simply on catch location.

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Commercial fisheries make important contributions to the economies of many countries, as well as to human well-being and social health but have also overexploited several fish species (FAO 2016). Ensuring the sustainable use of these valuable resources requires the development and implementation of fisheries management strategies, for which accurate status assessments (ie abundance, levels of mortality, etc) of fish

stocks (management units) are critical (Musick and Bonfil 2005). The development of successful fisheries management measures requires that reproductively isolated populations be assessed as independent stocks (Reiss *et al.* 2009); however, defining marine fish stocks is difficult, given that intermediate scenarios that fall between full random mating ("panmixia") and the total absence of genetic exchange among populations are frequent and difficult to discern. Such a scenario appears to apply to the iconic Atlantic bluefin tuna (ABFT, *Thunnus thynnus*), a highly migratory, large pelagic (open-ocean) fish that inhabits the North Atlantic Ocean and adjacent seas (Mather *et al.* 1995; Fromentin and Powers 2005), and whose sustainable management is a priority due to high demand in the expanding global fish market (Sissenwine and Pearce 2017).

Since the early 1980s, management of ABFT has presumed that there are two distinct stocks, separated at the 45°W meridian, based on the recognition of two main ABFT spawning grounds – the Gulf of Mexico and the Mediterranean Sea (Fromentin and Powers 2005) – and the assumption that there is no mixing or only low levels of mixing between the two stocks. Yet more recent tagging surveys (Lutcavage *et al.* 1999; Galuardi *et al.* 2010; Arregui *et al.* 2018) and analyses of otolith (ear stone) chemistry (Rooker *et al.* 2008, 2014) have challenged this management delineation by demonstrating regular and frequent trans-Atlantic migrations of ABFT adults, while also suggesting that individuals return to their place of birth to spawn (Block *et al.* 2005; Rooker *et al.* 2014). This behavior, termed "natal homing", suggests that ABFT should instead be managed as a mixed-stock fishery (ie one composed of spatiotemporally defined aggregations of individuals from different biological

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populations), which would require that individuals harvested in the mixing areas be assigned to their birth location.

Genetic analyses could provide decisive evidence of natal homing in ABFT, but such studies performed to date have been based on a small number of molecular markers that do not allow for the development of an origin traceability tool (Alvarado Bremer *et al.* 2005; Carlsson *et al.* 2007; Boustany *et al.* 2008); moreover, genetic studies have not taken into consideration a recently discovered potential alternative spawning location in the Northwest Atlantic Ocean (Richardson *et al.* 2016). The lack of genome-wide evidence in support of the natal homing hypothesis has prevented the development of a standardized, reliable, and cost-effective origin traceability tool, which is crucial for implementing a mixed-stock management approach for ABFT.

We performed population genetic analyses based on hundreds of reference samples (ie larvae and young of the year [YoY], both assumed to be found at or close to the area where they were spawned) and thousands of genome-wide single-nucleotide polymorphism (SNP) markers. Our analyses produced robust, direct genetic evidence of natal homing in ABFT; on the basis of those results, we then derived an origin traceability tool that we used to map the natal origin of 1000 ABFT individuals harvested from the mixing areas throughout the Atlantic Ocean, as well as of larvae and YoY from areas beyond the Gulf of Mexico and Mediterranean Sea, including larvae recently discovered in the Slope Sea (a segment of the Northwest Atlantic Ocean located approximately between the Gulf Stream to the south and the North American continental shelf to the north and west) (Richardson *et al.* 2016). This accurate and cost-effective origin assignment tool allows ABFT catches to be assigned to one of two biologically meaningful units, so that accurate stock assessments can be performed on each, enabling the development of an efficient and sustainable ABFT management strategy.

Methods

Detailed descriptions and a schematic representation of the samples used, methodological procedures, and approach that we followed throughout the study are presented in WebPanel 1. Samples from ABFT larvae, YoY, juveniles, and medium-to-large adults were obtained from scientific surveys and commercial fisheries operating throughout the species' known geographic distribution, including spawning grounds (WebTables 1–5). Genomic DNA was extracted from tissue or larvae samples and used to generate and sequence restriction-site-associated DNA (RAD-seq) libraries. Generated RAD-tags were quality filtered and used for SNP discovery and genotyping. Approximately 10,000 SNPs that passed quality filters and 204 samples (26 from the Gulf of Mexico; 13 from the Slope Sea; and 68, 48, and 49 from the Western, Central, and Eastern Mediterranean, respectively) were used for deciphering ABFT population structure based on principal component analyses (PCA) and Bayesian clustering of individuals into potential

ancestral populations. Based on the populations identified in the PCA and Bayesian clustering analyses, SNPs were ranked according to their discrimination level (degree of differentiation among populations), and the 230 most discriminant SNPs (WebTable 6) were genotyped in a new set of samples for technical and biological validation. From these, the 96 most discriminant SNPs (WebTable 7) were then genotyped in an additional set of known-origin reference samples to assess their assignment power as a percentage of correctly assigned samples. All reference samples of known origin genotyped for this final set of 96 SNPs constituted a baseline of 646 samples that was used for assignment of 940 adults of unknown origin collected at feeding aggregations, and of 21 larvae and YoY collected within or near potential spawning grounds.

Results

Direct genetic evidence of natal homing in ABFT

Our population genetic analyses, which were based on thousands of genome-wide SNP markers discovered and genotyped through RAD-seq (see WebPanel 2 for details) from more than 200 ABFT larvae and small YoY, revealed differentiation among Northwest Atlantic (including Gulf of Mexico and Slope Sea) and Mediterranean Sea locations (Figure 1). In the Bayesian analysis, despite all individuals exhibiting contributions from each of the two hypothetical ancestral populations, samples generally clustered by assumed stock, and the average probability of belonging to one of the two hypothetical ancestral populations was significantly different between the Northwest Atlantic and the Mediterranean Sea samples ($P = 2.2 \times 10^{-16}$). This genetic differentiation between the two main spawning grounds, coupled with the reported extensive trans-Atlantic migrations of ABFT, supports the natal homing hypothesis. On each side of the Atlantic Ocean, distinct patterns of genetic differentiation emerged. The Mediterranean Sea samples were genetically indistinguishable, supporting current paradigms (Arrizabalaga *et al.* 2018) and contradicting previous findings based on analysis involving only a few molecular markers (Carlsson *et al.* 2007; Boustany *et al.* 2008; Riccioni *et al.* 2010). In contrast, the Gulf of Mexico larvae and the Slope Sea YoY exhibited genetic differentiation in both the Bayesian analyses (their average probabilities of belonging to one of the two hypothetical ancestral populations differ; $P = 0.047$) and the PCAs (several Slope Sea samples overlapped with Mediterranean Sea samples); these results underscore the need for further research to decipher the western, eastern, or mixed population of origin of Slope Sea larvae.

Development of an origin traceability tool

The confirmation of natal homing by our population genetic analyses enabled the development of an accurate and operational origin traceability tool. We selected and validated

a subset of stock-differentiating SNPs (see WebPanel 3 for details) and included the 96 most discriminant SNPs in an origin traceability panel. Panel validation conducted on a reference set of samples excluded from SNP discovery or selection resulted in 81% and 83% of the Gulf of Mexico and Mediterranean Sea origin samples being correctly assigned, 10% and 2% incorrectly assigned, and 9% and 15% unassigned, respectively (Figure 2a). Despite the good performance of our assignment panel (89% and 98% of the samples with an assignment score above 80% were correctly assigned to the Gulf of Mexico or Mediterranean Sea, respectively) compared to previous endeavors (Puncher *et al.* 2018), there were samples that appeared to originate from a different region than that where they were collected, which was consistent with the pattern observed in the PCA of allele frequencies of our baseline samples (Figure 2b). Interestingly, the 95% confidence ellipses of each spawning component showed lower overlap as compared to those observed using otolith chemistry (Rooker *et al.* 2008, 2014), suggesting that SNP markers have higher discriminant power for assigning ABFT origin.

Similar to otolith chemistry analyses, origin traceability based on SNPs was more effective for samples collected in the Mediterranean Sea than for samples collected in the western Atlantic, and was not 100% in either case. This could be because a fraction of individuals may have spawned in a different region from that in which they were born, which, due to the larger biomass of the eastern stock (estimated to be 10 times as large as the western stock biomass), would mean more individuals of Mediterranean origin were not returning to their natal area. Another explanation could be the limited number of Gulf of Mexico and Slope Sea samples used for the first SNP selection, which may not have been large enough to capture the full diversity in the Northwest Atlantic. Finally, it may also be that our SNP panel was incapable of capturing the entire genetic diversity, thereby restricting our ability to perform a perfect assignment due to a limited number of molecular markers, which could be possible if the separation of stocks is recent (Alvarado Bremer *et al.* 2005). If this were the case, then increasing the SNP number would lead to an increase in assignment power. We observed, however, that maximum assignment power was attained with as few as 36 SNPs, when these were selected from among the most discriminant of the 96 SNPs (Figure 2c).

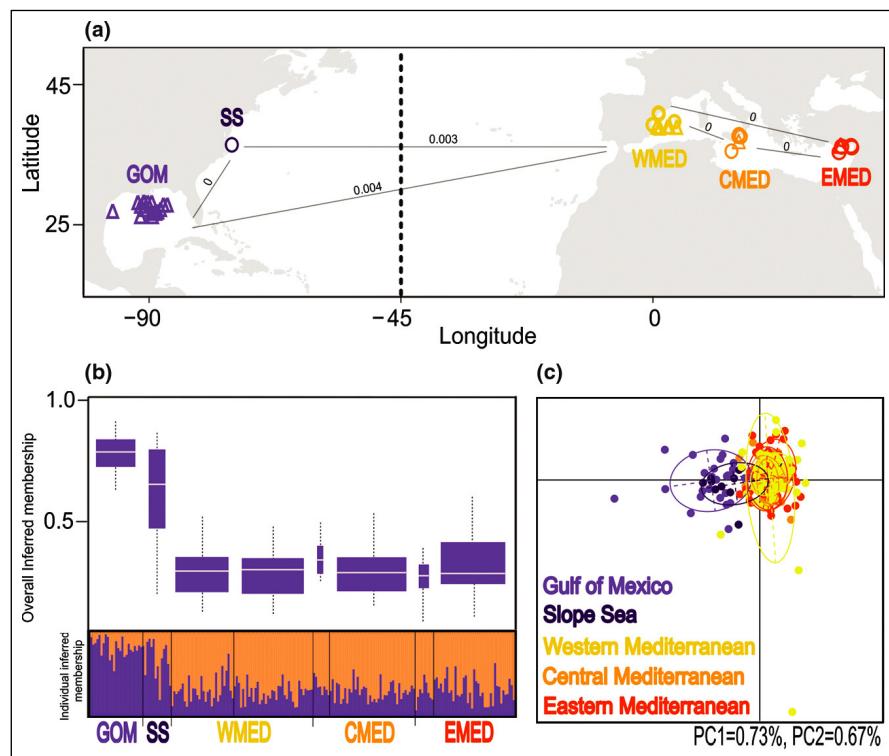


Figure 1. Genetic differentiation among main spawning grounds. (a) Map showing the stock delimitation meridian (dashed vertical line) and depicting the locations where reference samples used in our population genetic analyses were collected; F_{ST} values (rounded to three decimals) among each pair are indicated; triangles denote larvae and circles denote young of the year (YoY). (b) The bottom panel depicts a graphical representation of the Bayesian clustering approach, where each bar represents an individual and each color represents its inferred membership to each of two potential ancestral populations ($K = 2$). In the top panel, boxplots – sized proportionally to the number of individuals – illustrate the median (horizontal line within box), the 25th and 75th percentile (values within box), and the largest and smallest values within the 1.5-times interquartile range above the 75th percentile (vertical line above box) or below the 25th percentile (vertical line below box) of the assignment of individuals from each location to one of two hypothetical ancestral populations; for the different Mediterranean locations, larvae and YoY are situated left and right, respectively, of the black line separating individuals from one location. (c) Principal component analysis (PCA) of allele frequencies. The first two principal components are shown; each dot represents one sample colored according to its area of origin. Ovals represent 95% inertia ellipses. Based on catalog 1 (see WebFigure 1, WebFigure 2, and WebTable 8 for all catalogs).

Mapping the origin of ABFT mixing aggregates

Origin assignment using the newly developed 96 SNP panel suggested that most individuals caught in each area were born in the spawning ground closest to where they were caught (Figure 3). The proportion of western origin ABFT in eastern fishing grounds varied between 0–9% (average of 4%), and the proportion of eastern-origin ABFT in western fishing grounds varied between 23–56% (average of 37%). Individuals caught in Norway (close to the northern distribution limit of the species) and Mauritania (where ABFT observations are very rare), whose stock of origin has not been studied previously, seem to be mainly of Mediterranean Sea origin. The proportion of western-origin bluefin in other Eastern regions (Central Atlantic, Bay of Biscay, Gulf of Cadiz,

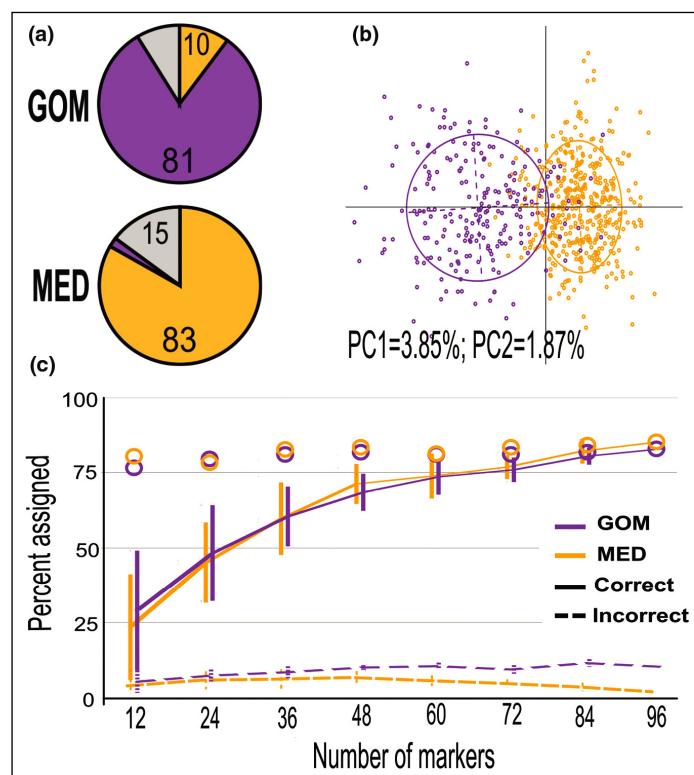


Figure 2. Composition of the genetic baseline and origin assignment success rates. (a) Percentages of samples assigned to where they were caught, for reference samples captured in the Gulf of Mexico (GOM) and the Mediterranean (MED); purple indicates Gulf of Mexico origin, orange indicates Mediterranean origin, and gray indicates unassigned samples. (b) PCA of allele frequencies of the Gulf of Mexico (purple) and Mediterranean (orange) individuals included in the baseline. The first two principal components of the PCA are shown; each dot represents one sample, and ovals represent 95% inertia ellipses. (c) Progression of the percentage of correctly or incorrectly assigned Gulf of Mexico (purple) and Mediterranean (orange) caught samples as the number of markers (SNPs) used increases. Vertical bars indicate standard deviation; open circles indicate correct assignment rates for the most discriminant subsets of SNPs. Panels (a) and (c) were calculated for an 80% assignment score threshold; see WebFigure 3 for 70% and 90% assignment scores, as well as for sensitivity and specificity analyses of each threshold.

Strait of Gibraltar, Morocco, and Canary Islands) is comparable with previous estimates using otolith chemistry (Rooker *et al.* 2014). The origin of individuals caught west of the 45°W meridian is more variable between regions, and the proportion of Mediterranean Sea–origin tunas is highest in the Central Atlantic and lowest in the Newfoundland–Labrador area. Notably, the Gulf of St Lawrence shows an unexpectedly high proportion of ABFT individuals of Mediterranean Sea origin, which may be due to the greater abundance of the eastern population, such that even relatively low migration rates can result in a high proportion of Mediterranean Sea–origin fish in this area.

For the first time, larvae and YoY caught in two potential spawning areas outside the Mediterranean Sea and Gulf of Mexico have been genetically analyzed. All confidently

assigned Canary Islands individuals to Mediterranean Sea origin; although the Canary Islands have previously been suggested as a potential spawning area (Mather *et al.* 1995), it is unclear if the YoY collected here were from natal sites in the eastern Atlantic Ocean or were migrants from the Mediterranean Sea. On the other hand, larvae caught in the Slope Sea were assigned to both of the main spawning areas, but given the distance to the two main spawning grounds and estimated larval age (less than 4 days), it is not possible that these larvae were spawned in the Gulf of Mexico or the Mediterranean Sea. Instead, these results suggest more complex scenarios: (1) Slope Sea spawners are part of a single population that includes fish born in the Mediterranean Sea; (2) Slope Sea spawners are part of a single population that includes fish born in the Gulf of Mexico; (3) Slope Sea spawners form an independent population; (4) individuals from the Gulf of Mexico and the Mediterranean Sea use this area independently as an alternative spawning site; or (5) individuals from the Gulf of Mexico and Mediterranean Sea interbreed in this area. Further targeted genetic studies are needed to test these hypotheses and shed light on the contribution of the Slope Sea and other potential spawning areas in the Atlantic.

Discussion

The assessment and management of ABFT stocks have been hindered for decades by an incomplete understanding of the species' complex migratory patterns. Spatial dynamics is especially important for the assessment and management of the western stock (Morse *et al.* 2018), which is estimated to be an order of magnitude smaller than the eastern stock. Given the extensive and interannually variable mixing across the Atlantic (Galuardi *et al.* 2010; Arregui *et al.* 2018; Arrizabalaga *et al.* 2018), the hypothesis behind the current management approach, where fish caught west of the 45°W meridian are assigned as being of western origin and vice versa, is not valid. Instead, an appropriate management approach should rely on a tool that accurately and cost-effectively assigns ABFT catches to a given stock based on where they were spawned and not on where they were caught.

The origin assignment panel presented here allows for the annual assignment of catches to population of origin within the same turnaround time that the International Commission for the Conservation of Atlantic Tunas (ICCAT) currently provides for annual catch estimates, enabling timely catch reporting in terms of biologically meaningful stock units rather than broad spatial areas. Our origin traceability panel is based on a robust sample baseline, provides accurate origin assignment, and is cost-effective (less than US\$10 per sample; Campbell *et al.* 2014). Moreover, this non-invasive tool can be operationalized to screen the origin of international catches by following a simple sampling protocol and without affecting the market value of the fish. This facilitates mixing-based assessment approaches (Taylor *et al.* 2011) that can capture stock-specific productivity dynamics and tailor management strategies for each stock. In

addition, while the current management regime allows for only area-based quotas to be implemented, which is ineffective for ensuring sustainable population harvesting, an operational implementation of the genetic tool developed here would make population-specific quotas possible, which are critical for effective management of ABFT. This new genetic tool comes at an opportune time, as alternative management strategies are currently under consideration by ICCAT (Carruthers *et al.* 2016) and could include approaches based on monitoring of population-specific exploitation rates (Bradbury *et al.* 2014). This scientific approach is relevant for ABFT fishery managers, who will now be able to see their management actions having the expected impacts on ABFT populations.

Our research also represents a promising avenue for other mixed-stock fisheries around the world. Unfortunately, fish stocks often do not correspond to true biological populations (Reiss *et al.* 2009) and, consequently, management actions do not always have the expected impacts (eg recovery of overfished stocks) and mismanaged populations remain at risk. Developing genetic tools to assign catches to the correct population will support more effective fisheries management worldwide.

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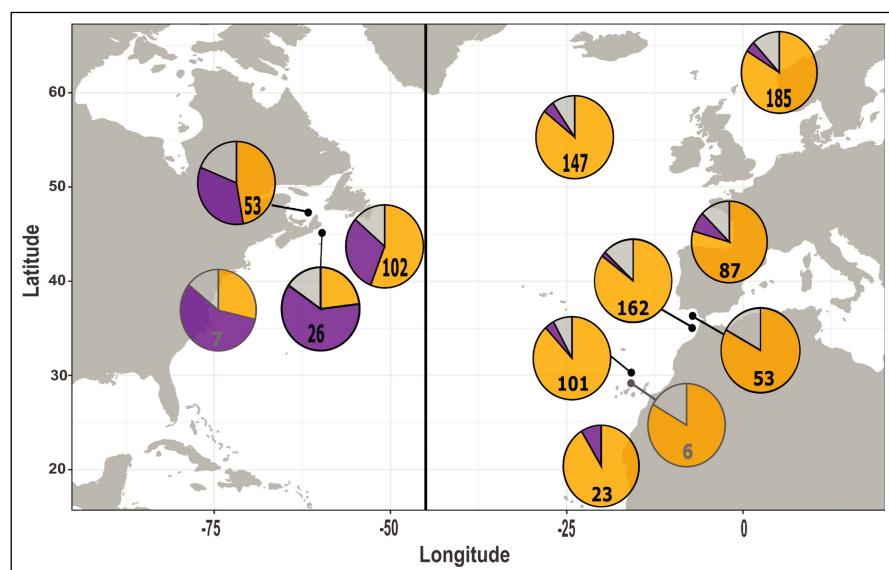


Figure 3. Origin assignment of mixing aggregates and reference samples from outside the main spawning grounds. Proportion of samples assigned to the Mediterranean (orange) or Gulf of Mexico (purple); gray slices denote unassigned samples. Black outline indicates mixing aggregates; gray outline indicates Slope Sea larvae and Canary Islands YoY. Values indicate the number of samples analyzed per location.

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■ Supporting Information

Additional, web-only material may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/fee.2090/suppinfo>



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UV biofluorescence in swallowtail butterfly larvae

Animals communicate through various signals, including those produced by bioluminescence. Not all organisms are able to luminesce, but some taxonomic groups such as fish have a higher proportion of bioluminescent (ie light-emitting) species, while numerous arthropods are biofluorescent (ie reflecting light in a different part of the spectrum). Certain insects are bioluminescent while others possess a different attribute: they reflect light in the ultraviolet range and qualify as ultraviolet (UV) biofluorescent, although the purpose of this trait is far from clear.

The ultramarine spots on the hind wings of adult Asian swallowtails (*Papilio xuthus*) exhibit UV biofluorescence, but we found no previous reports of biofluorescence in their caterpillars. During night surveys on October 6, 2018, in Namsan Park, Seoul, South Korea, we observed an Asian swallowtail caterpillar under normal light and under black light (390–395 nanometers; Ultraviolet Blacklight Flashlight 51 LED, Escolite; Chicago, USA). The caterpillar's entire body showed biofluorescence, except for the dark markings.

Birds are the main predators of caterpillars and typically forage at dawn, when the light shifts to shorter wavelengths, and towards the UV range. UV-reflecting patterns on wings of the woodland brown butterfly (*Lopinga achine*) decrease predation by birds because of the



deflective role of marginal eyespots, and UV reflectance by the *P. xuthus* caterpillar may be another example of anti-predatory morphology. Alternatively, as UV reflectance in adults is acquired through the caterpillar's diet in pterins, a molecule used for pigments, UV reflectance in the caterpillars may be a side effect of pterin-rich diets to ensure fluorescence in adults.

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