

# From scales to armor: Scale losses and trunk bony plate gains in ray-finned fishes

Alexandre Lemopoulos<sup>1</sup> and Juan I. Montoya-Burgos<sup>1,2,3</sup> 

<sup>1</sup>Department of Genetics and Evolution, University of Geneva, Geneva, Switzerland

<sup>2</sup>iGE3 Institute of Genetics and Genomics of Geneva, Geneva, Switzerland

<sup>3</sup>E-mail: Juan.Montoya@unige.ch

Received November 20, 2020

Accepted February 4, 2021

Actinopterygians (ray-finned fishes) are the most diversified group of vertebrates and are characterized by a variety of protective structures covering their integument, the evolution of which has intrigued biologists for decades. Paleontological records showed that the first mineralized vertebrate skeleton was composed of dermal bony plates covering the body, including odontogenic and skeletogenic components. Later in evolution, the exoskeleton of actinopterygian's trunk was composed of scale structures. Although scales are nowadays a widespread integument cover, some contemporary lineages do not have scales but bony plates covering their trunk, whereas other lineages are devoid of any such structures. To understand the evolution of the integument coverage and particularly the transition between different structures, we investigated the pattern of scale loss events along with actinopterygian evolution and addressed the functional relationship between the scaleless phenotype and the ecology of fishes. Furthermore, we examined whether the emergence of trunk bony plates was dependent over the presence or absence of scales. To this aim, we used two recently published actinopterygian phylogenies, one including > 11,600 species, and by using stochastic mapping and Bayesian methods, we inferred scale loss events and trunk bony plate acquisitions. Our results reveal that a scaled integument is the most frequent state in actinopterygians, but multiple independent scale loss events occurred along their phylogeny with essentially no scale re-acquisition. Based on linear mixed models, we found evidence supporting that after a scale loss event, fishes tend to change their ecology and adopt a benthic lifestyle. Furthermore, we show that trunk bony plates appeared independently multiple times along the phylogeny. By using fitted likelihood models for character evolution, we show that trunk bony plate acquisitions were dependent on a previous scale loss event. Overall, our findings support the hypothesis that integument cover is a key evolutionary trait underlying actinopterygian radiation.

**KEY WORDS:** Actinopterygians, ancestral state, functional innovation, phylogeny, skeleton evolution, integument.

## Impact Summary

Ray-finned fishes (actinopterygians) are the most diverse vertebrate group in the world. The majority of these fishes possess scales as a protective shield covering their trunk. However, several lineages display a body armor composed of trunk bony plates or are devoid of any protective structures. The diversity and the transitions between different integument coverage types have not been previously studied in an evolutionary framework. Here, we investigate which integument protection

was present at the origin of ray-finned fishes and how new protective structures emerged and evolved through time.

We show that a scaled integument was the most widespread state along with ray-finned fish evolution, yet scale losses occurred multiple times independently, while acquiring scales again almost never happened. Moreover, we reveal that scaleless integuments most probably led species to change their ecology and colonize the floors of oceans and water bodies. The functional advantages of a scaleless integument in a benthic environment are yet to be demonstrated, but the increased cutaneous respiration could be an explanation.

We show that trunk bony plates also emerged independently multiple times along the evolution of ray-finned fishes but these armors protecting the trunk can only appear after a scale loss event. Therefore, while the acquisitions of trunk bony plates are phylogenetically independent, they need a “common ground” to emerge. All together, our findings provide evidence that the integument covers have contributed to the outstanding diversification of ray-finned fishes.

Ray-finned fishes (Actinopterygii) represent the most diversified vertebrate lineage in the world, with more than 33 thousand described species (Alfaro 2018). One of the most prominent features among Actinopterygii representatives is the presence of scales in their trunk integument forming a protective layer. Scales can display various shapes and structures, as they can contain different compounds and differ in histological characteristics (Moyle and Cech 2004). The diversity of scales has created some confusion in the scientific community, because different skeletal elements have been referred to as scales despite being of different origin (Schultze 2018). Yet, given the great diversity and the complexity of these structures, a consensus over their nomenclature and classification still needs to be established based on a comprehensive understanding of their evolutionary origin (Sire et al. 2009; Vickaryous and Sire 2009). In this study, we primarily focus on two categories of mineralized structures developing within the integument of actinopterygians, micromeric scales, and macromeric trunk bony plates.

Scales, as differentiated micromeric dermal skeletal elements (*sensu* Sire 2003 and Sire et al. 2009) were present in the ancestral lineage that gave rise to Actinopterygii and Sarcopterygii (Sire et al. 2009). Therefore, scales are considered a plesiomorphic trait for ray-finned fishes and today the majority of them possess some type of scales (Gemballa and Bartsch 2002; Sire et al. 2009). Based on different histological and morphological properties, scales have been classified in two main groups: ganoid scales (in Protopterygidae (bichirs) and Lepisosteiformes (gars) [Meunier and Brito 2004; Ichiro et al. 2013]) and elasmoid scales (in the majority of actinopterygian lineages; e.g. Sire et al. 1997; Mongera and Nüsslein-Volhard 2013). All scales possess a bony layer (e.g., bony-ridge, lammellar bone) in their structure (Benthon 2004; Moyle and Cech 2004; Zhu et al. 2012). Thus, scales are a bony structure covered with a scale-specific odontogenic-like tissue, in general. The nature of the odontogenic-like cover and the scale organization then define the type of scale (e.g., ganoin in ganoid scales; Ichiro et al. 2013). Therefore, two components are in general necessary for the formation of a scale: (a) a bone micromeric structure; and (b) an odontogenic-like cover tissue that is scale-specific (but this tissue is sometimes reduced or even absent).

Trunk bony plates (TBP) represent another type of integument protection, which is present in some extant actinopterygians. The origin of TBP can be traced back to the first vertebrate mineralized skeleton, which was composed of TBP covered with an odontogenic tissue (Keating and Donoghue 2016). Independently of their evolutionary history, TBP *sensu lato* can be differentiated from scales as they are macromeric integument elements composed of bone only (i.e., lacking the odontogenic-like cover). TBP, as macromeric integument structures, reappeared in specific actinopterygian lineages. For instance, the iconic seahorse (Syngnathidae) exoskeleton is made of dermal bony plates covering the entire body (Lees et al. 2012; Porter et al. 2013). Other examples are the Callichthyidae and the Loricariidae, two species-rich families of Neotropical catfishes, that have their trunks covered with TBP (Sire 1993; Covain et al. 2016; Rivera-Rivera and Montoya-Burgos 2017). Interestingly, micromeric scales and macromeric TBP seem to be mutually exclusive as no extant fish displaying both exoskeletal structures in the trunk has been reported to date.

Despite the widespread occurrence of protective elements in the integument of fishes, several lineages within actinopterygians display a naked skin, that is, devoid of any scales or any other protective structures. Whether the lack of scales in several ray-finned fishes is a result of independent scale loss events rather than multiple independent appearances of scales has not been formally assessed. Nevertheless, the putative selective advantage of a scaleless skin is compelling. Some functional advantages have been suggested, such as an increased cutaneous respiration (Park and Kom 1999; Park 2002), or a relatively higher expression of immune genes after a parasitic infection as measured in scaled *versus* scaleless skin regions of salmon (Holm et al. 2017). Yet, the extent of the advantages and disadvantages of having a scaleless integument is unclear. Nevertheless, we observed that scaleless fishes belonging to different lineages tend to have a benthic habitat preference. In addition, they present a similar overall morphology corresponding to the one typically found in bottom-dwelling species (e.g., inferior mouth, flattened abdomen, or body) according to the classification of Moyle and Cech (2004). Whether a relationship between a scaleless integument and habitat preference exists in ray-finned fishes needs to be examined further.

In this study, we investigated the drivers of trunk integument evolution in actinopterygians. We first hypothesized that the loss of scales may be related to a bottom-dwelling lifestyle, as this state could result in functional advantages in a benthic environment. Second, as apparently scales on the skin cannot co-occur with TBP in the same fish species, we tested the hypothesis that the loss of scales is an evolutionary prerequisite for the (re)emergence of TBP. To test these hypotheses, we inferred the evolutionary history of the emergence and disappearance of scales and TBP along with the evolution of actinopterygians. To this aim, we used two recently published ray-finned fishes

phylogenies, one containing 304 species (Hughes et al. 2018) and the other 11,638 species (Rabosky et al. 2018). The magnitude of this dataset allowed us to have a precise view on actinopterygian evolution. We collected data regarding habitat preference and trunk integument characteristics for each species of the phylogenies. We then performed ancestral state reconstructions and we investigated the associations between traits using methods of linear regression for binary data and likelihood model fitting for character evolution.

## Materials and Methods

### PHYLOGENY

To perform the ancestral state reconstruction analyses and to account for the possible effect of (i) variation in the phylogenetic inferences (phylogenetic uncertainty) and (ii) phylogenetic relatedness of the traits in the correlation analyses, we used two recently published ray-finned fishes phylogenies (Hughes et al. 2018; Rabosky et al. 2018).

Hughes et al. (2018) published a robust and well-resolved phylogeny obtained by using 1105 orthologous exons of 305 species representing all actinopterygian lineages, including most of the lineages displaying the traits examined in this study. One species, *Xenopus tropicalis*, used as an outgroup in Hughes et al. (2018) phylogeny was excluded from our analysis as it was irrelevant in the context of our study. The phylogeny by Rabosky et al. (2018) was reconstructed based on a 27 genes alignment for 11,638 species (with a substantial amount of missing data, see Rabosky et al. 2018). It is currently the most complete phylogeny as it contains almost all actinopterygian species.

### INTEGUMENT CHARACTERISTICS, MORPHOLOGY, AND HABITAT PREFERENCE

Information about the traits displayed by fish species was collected in two books (Moyle and Cech 2004; Nelson et al. 2016) and in Fishbase (Froese and Pauly 2011). When information was lacking or unclear in these three main sources, species characteristics were extracted from the specialized literature (Table S1 and Table S2). As the presence of scales is the ancestral trait of actinopterygians (Friedman and Brazeau 2010; Qu et al. 2013) we reported evidence for changes of traits, such as the absence of scales in the species or the presence of trunk bony plates (Table S1 for the 304 species dataset and Table S2 for the 11,638 species dataset).

To assess the link between absence of scales and habitat preference, we used as a baseline the classification of Moyle and Cech (2004) that links morphology to habitat preference. They described 10 different types of morphology-habitat associations, classified into five main categories. Out of these categories, four include fishes with middle or surface water habitat preference,

while one category consists of fishes with bottom habitat preference (bottom-dwellers). According to Moyle and Cech (2004), this bottom-dwelling category contains five types of morphologies: (1) bottom-rovers (e.g., Siluriformes), (2) bottom-clingers (e.g., Cottidae), (3) bottom-hiders (e.g., some Percidae), (4) flatfish (e.g., Pleuronectiformes), and (5) rattail (e.g., Macrouridae). Here, we individually assessed and assigned each species present in the phylogenies to either the bottom-dwelling category or to the non-bottom-dwelling super-category. In addition, we used available literature for refining the species habitat preference in ambiguous cases. For instance, even though Moyle and Cech (2014) do not consider eel-like fish as bottom-dwellers, some eel-like species are bottom-associated such as swamp eels (*Synbranchus marmoratus*). The corrected species allocation to the bottom-dwelling category or to the non-bottom-dwelling super-category is presented in Table S1 for the 304 species dataset (Hughes et al. 2018) and in Table S2 for the 11,638 species dataset (Rabosky et al. 2018).

To differentiate TBP from scales we used the description by Sire and Huysseune (2003). Based on different phylogenetic, developmental and histological characters, they described 10 different dermal skeletal elements in fish trunks, which can be subdivided into (i) large macromeric bony plates and (ii) small micromeric scale-like elements. Trunk macromeric bony plates include postcranial dermal bones, and scutes (trunk bony plates specific to some Neotropical catfish), which we refer to as TBP. Trunk micromeric scale-like structures include odontodes (superficial structure with dental tissues), ganoid scales (of polypterids and lepisosteids) and elasmoid scales and they were here referred to as scales. In our study, we did not consider oral and extra-oral teeth or denticles as trunk scale-like elements as they represent more complex structures including dentine, enamel-like covers, a pulp cavity, a particular attachment to the underlying bone, and an innervation in most cases. For each species, the integument characteristics are presented in Table S1.

### ANCESTRAL STATE RECONSTRUCTION

We performed two different ancestral state reconstructions for the presence / absence of scales and for the presence / absence of TBP. We first used a stochastic mapping approach for morphological characters (Huelsenbeck et al. 2003). We used the `make.simmap` function in the `phytools` package v.06.99 (Revell 2012) in R environment v. 3.6.1. To select the best model of transition rate, we compared AIC scores between the equal rate (ER) and all rates different (ARD) models (Table S3). The Q matrix for transition rates was sampled based on posterior probabilities after 250'000 generations (Q = "mcmc") with a burnin phase of 10,000 generations. Prior probability distributions were set empirically with the option `prior = use.empirical = true`.

Second, we performed ancestral reconstructions using Bayestrait 2.0 (Pagel et al. 2004). For these reconstructions, we compared uniform and exponential reverse-jumping hyperprior (Pagel 2004). By comparing likelihood scores obtained following the stepping stone approach (100 to 1000), we used the logBF factor to identify the best model for each scenario (Pagel et al. 2004; Table S3). To constrain jump acceptance rates for each model between 0.2 and 0.4, we used hyperpriors ranging from 0 to 30 as recommended by the software manual. We performed respectively 50,000,000 MCMC iterations for the 304 species phylogeny and 10,000,000 iterations for the 11,638 species phylogeny. Trees and node probabilities were visualized using Treegraph 2 (Stöver and Müller 2010).

Finally, to evaluate how phylogenetic uncertainty could influence the ancestral reconstruction, in addition to working with two different datasets (Hughes et al. 2018; and Rabosky et al. 2018), we performed a multi-tree ancestral state reconstruction using the 304 species dataset of Hughes et al. (2018). We performed a phylogenetic inference with Exabayes (Aberer et al. 2014) based on the protein super-alignment provided by Hughes et al. (2018). We used the Hughes et al. (2018) best phylogeny as a starting tree in Exabayes and the other parameters were set to default. Iterations were executed until convergence was reached and visualised through Tracer v.1.7 (Rambaut et al. 2018). From the output of this analysis, we used a subset of 1000 trees to perform a multi-tree ancestral state reconstruction in Bayestrait 2.0. The parameters used were the same as the ones used on the single best tree analysis (Table S3).

### ASSOCIATION AND DEPENDENCY ANALYSES

For the scaled/scaleless fish dataset and the presence/absence of TBP dataset, we calculated the  $D$  value, which is an index that indicates whether binary traits evolve independently or evolve according to the phylogeny under a Brownian motion model (Fritz and Purvis 2010). Thus, this value indicates to what extent the evolution of the traits is linked to the phylogeny (0 = no relationship; 1 = full dependency). We calculated this value using the phylo.D function in caper library v.1.0.1 (Orme et al. 2013).

To test the hypothesis that the scaleless phenotype is associated with a benthic habitat preference, we performed a linear regression for binary (discrete) data using the binaryPGLMM function in ape package v.5.3 (Paradis et al. 2004). Using this function, we tested whether the presence/absence of scales was explained by the habitat preference, and accounting for the phylogeny (Scale.State~Ecology+[Phylogeny]). Parameters were set to default and convergence of the model was assessed using the build-in function.

To test the evolutionary relationship between scales and TBP, and more specifically whether the emergence of TBP was dependent over the absence of scales, we studied the association

between presence/absence of TBP and presence/absence of scales using the fitPagel function in the phytools package (Revell 2012). This function is designed to analyse the coevolution of two traits and the way they are linked over the course of time by providing a phylogeny as an input to the method.

## Results

### SCALE CONDITION AND HABITAT PREFERENCE

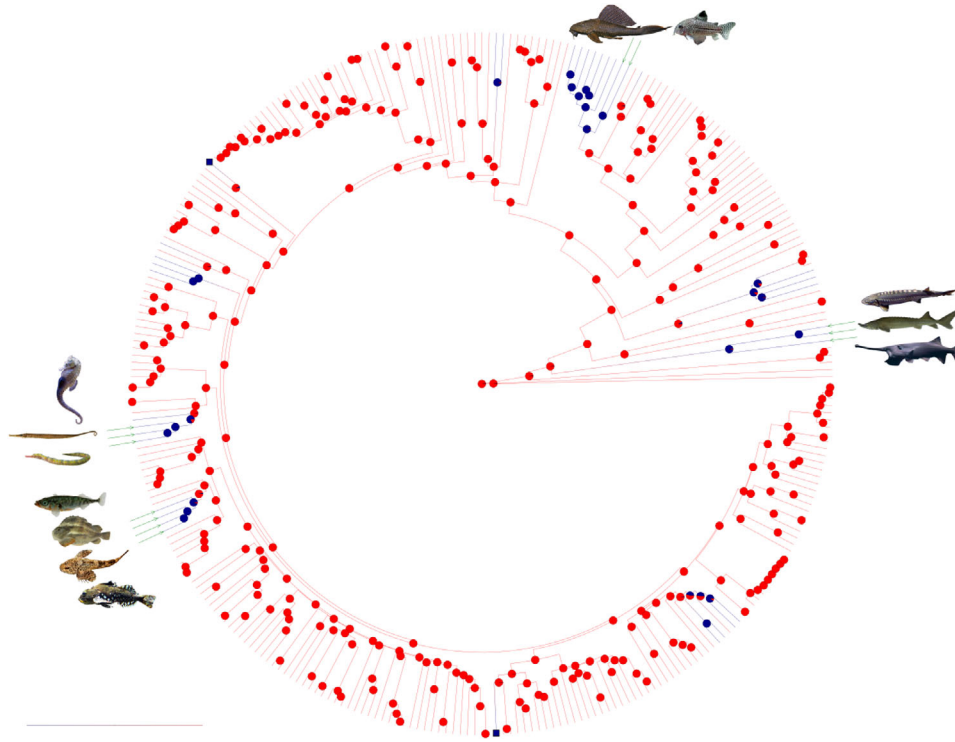
Out of the 304 species considered in the phylogeny of Hughes et al. (2018), we identified 38 species as being scaleless (Table S1). 70 species were considered as bottom-dwellers, and 234 as non-bottom-dwellers. Ancestral state reconstructions were virtually the same with both stochastic mapping (Fig. 1 and Fig. S1 for species names) and Bayesian methods (Fig. S2 and Fig. S3 for phylogenetic uncertainty). In both reconstructions, we identified 11 scale loss events. The phylogenetic index  $D$  was not significant (Table 1) indicating that scale loss events bear no phylogenetic signal, that is, they occurred independently in different parts of the phylogeny. One event of scale re-acquisition following a loss was also inferred, namely in the *Anguilla* genus. However, this re-acquisition was observed only in the stochastic mapping reconstruction (Fig. 1 and Fig. S1), not in the Bayestrait reconstruction (Fig. S2 and Fig. S3). Results were similar when taking phylogenetic uncertainty into account by analysing a set of 1000 trees with Bayestrait (Fig. S3). However, an additional scale re-acquisition event was inferred with this reconstruction. This event occurred in the Opisthognathidae family (Fig. S3).

In the 11,638 species dataset of Rabosky et al. (2018), we identified 2,310 species as scaleless and 4,169 as bottom-dwelling. In the ancestral state reconstruction, 32 and 43 scale loss events were inferred with the stochastic mapping method (Fig. 2 and Fig. S4 for species names) and the Bayestrait method (Fig. S5), respectively. As to scale acquisition, 10 and 13 events were inferred with the stochastic mapping (Fig. 2 and Fig. S4) and the Bayestrait (Fig. S5) methods, respectively. The phylogenetic index  $D$  was not significant, indicating that these trait changes are not phylogenetically linked (Table 1).

The linear regression analyses to test for the association between the scaleless state and habitat preference showed with both datasets that the scaleless state and a benthic lifestyle are tightly linked (Table 2). Thus, fish presenting a scaleless integument are potentially more likely to display a benthic habitat preference.

### TRUNK BONY PLATES EMERGE ON A SCALELESS INTEGUMENT

In the 304 species dataset, we identified 12 species displaying TBP, distributed over nine families. In both ancestral state reconstructions of presence/absence of TBP, stochastic mapping



**Figure 1.** Reconstruction through stochastic mapping of the scale presence/absence on a phylogenetic tree of 304 Actinopterygii species (modified from Hughes et al. 2018, see Fig. S1 for the detailed tree). Red clades correspond to scaled taxa, while blue color indicates lineages that underwent a scale loss event. Fish illustrations represent species displaying trunk dermal bony plates (TBP). The four distinct gains of TBP occurred in distant lineages, yet always after a scale loss event. On the right, three species of Acipenseriformes: *Polyodon spathula*, *Acipenser sinensis*, *Acipenser naccarii*; on top, two species of Siluriformes: *Corydoras julii*, *Pterygoplichthys pardalis*; on the left three species of Syngnathiformes: *Syngnathoides biaculeatus*, *Syngnathus scovelli*, *Hippocampus erectus* and on the bottom left, four species of Gasterosteiformes: *Gasterosteus aculeatus*, *Cyclopterus lumpus*, *Cottus rhenanus*, *Myoxocephalus scorpius*.

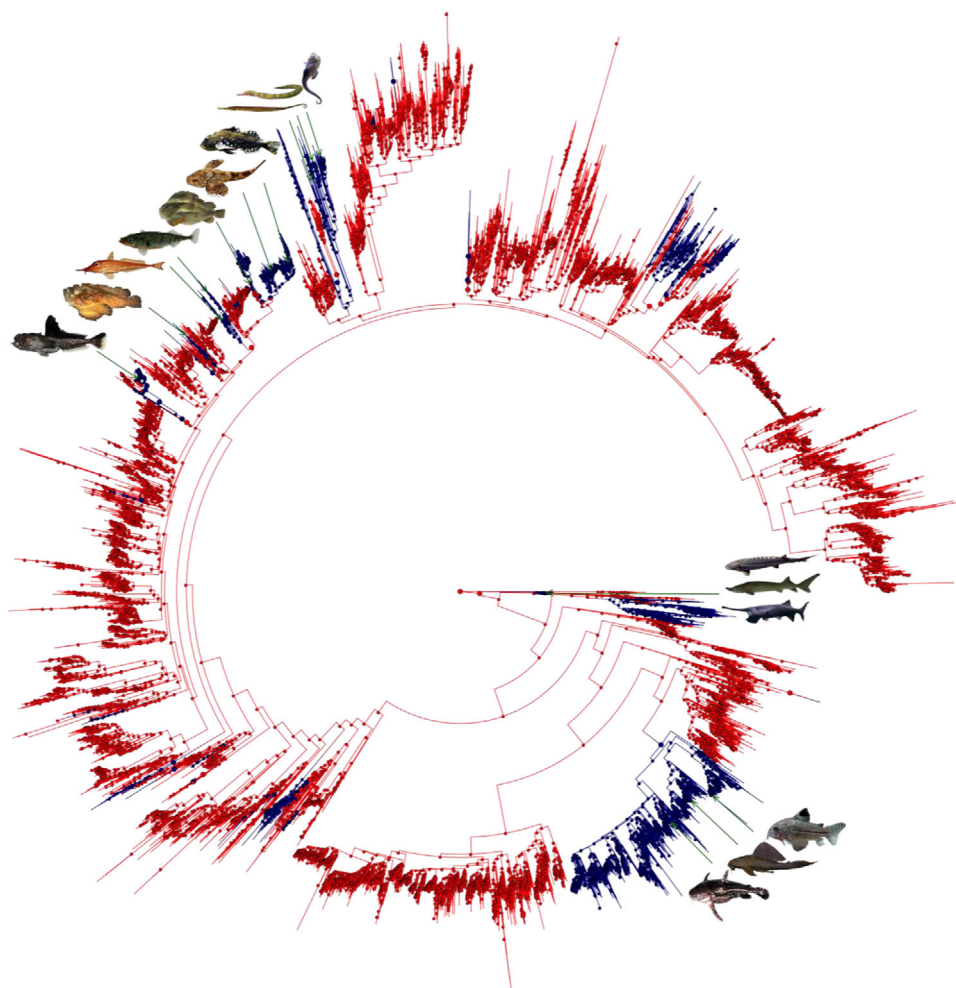
**Table 1.** The  $D$  statistics for the phylogenetic structure of two binary traits: presence / absence of scales; presence / absence of trunk bony plates (TBP). The two datasets were tested: 304 species (Hughes et al. 2018) and 11,638 species (Rabosky et al. 2018). Both traits evolve randomly across the phylogenetic trees and bear no phylogenetic information.

	Random phylogenetic structure (p-value)	Brownian phylogenetic structure ( $P$ -value)
Scales (304 species dataset)	<0.001	0.9907
Scales (11,638 species dataset)	0	1
TBP (304 species dataset)	<0.001	0.9547
TBP (11,638 species dataset)	0	1

(Fig. S6) and Bayestrait (Fig. S7), 4 events of TBP acquisition were inferred. In contrast, no loss of TBP was inferred. The phylogenetic index  $D$  was not significant (Table 1), meaning that TBP appeared independently in different parts of the phylogeny. Results were almost identical when taking phylogenetic uncertainty into account by using a set of 1000 trees (Fig. S8). Only

one additional TBP gain was identified in the Siluriformes order, but with a poor probability support.

In the 11,638 species dataset, 823 species displayed TBP (Table S2). The phylogenetic index  $D$  was also not significant (Table 1) for TBP appearance. In the stochastic mapping reconstruction (Fig. S9), 16 plate gains and 6 plate losses were



**Figure 2.** Ancestral trait reconstruction through stochastic mapping of the scale presence/absence on a phylogenetic tree of 11,638 Actinopterygii species (modified from Rabosky et al. 2018, see Fig. S5 for the detailed tree). Red clades indicate scaled taxa, while blue color correspond to scaleless taxa. Pictures correspond to species representing lineages displaying TBP. These are found in several unrelated lineages, yet always after a scale loss event. Species illustrated in Fig. 1 are also represented here, in addition to other species not included in the dataset of Fig. 1. On the top left, 10 fishes with TBP gains are illustrated. From left to right, one species of Perciformes: *Pogonophryne barsukovi* (first gain). Two species of Scorpaeniformes: *Synanceia verrucosa* and *Peristedion gracile* (second and third gains). Within Gasterosteiformes, four species illustrate the fourth (*Gasterosteus aculeatus*) and the fifth TBP gain (*Cyclopterus lumpus*, *Cottus rhenanus*, *Myoxocephalus Scorpius*). Three species of Syngnathiformes: *Syngnathoides biaculeatus*, *Syngnathus scovelli*, *Hippocampus erectus* represent the sixth gain. On the center right, three species of Acipenseriformes: *Polyodon spathula*, *Acipenser sinensis*, *Acipenser naccarii* illustrate a distinct TBP gain event. Finally, on the bottom right, three species of Siluriformes: *Corydoras julii*, *Pterygoplichthys pardalis*, and *Acanthodoras spinosissimus* are other examples of TBP gain.

**Table 2.** BinaryPglmm and FitPagssel test between different traits of actinopterygians: ecology versus scale phenotype; trunk bony plates (TBP) phenotype versus scale phenotype. The two datasets were tested: 304 species (Hughes et al. 2018) and 11,638 species (Rabosky et al. 2018).

	304 species dataset	11,638 species dataset
binaryPGLMM	4.788e-05	5.288e-14
P-value: ecology ~ scales		
R2	0.8089829	0.9906676
FitPagel P-value: TBP ~ scales	9.165e-5	6.149992e-25

identified, while 21 plate gains and 11 plate losses were identified in the Bayestrait reconstruction (Fig. S10).

We assessed whether TBP appearance depends on a specific integument condition using the fitPagel test. The results indicated that for both the 304 and 11,638 fish species datasets, the presence of TBP is dependent on a specific integument scaling state (Table 2). More precisely, TBP have a significant and strong tendency to appear in a scaleless integument.

## Discussion

Ray-finned fishes form the most species-rich group of extant vertebrates, and the reasons of their evolutionary radiation remain unclear. A number of functional innovations have been put forward to explain the wide radiation in Acanthomorpha, the main actinopterygian subgroup (Wainwright and Longo 2017), but the protection provided by their scaled exoskeleton is rarely considered. Yet, material engineers have demonstrated the mechanical and protective properties of fish scales (e.g., Zu et al. 2012), providing empirical evidence supporting that scales could be another functional innovation explaining the radiation of ray-finned fishes in the aquatic environment.

### ABSENCE OF SCALES IS ASSOCIATED TO HABITAT PREFERENCE

Although a scaled integument is one of the main characteristics of ray-finned fishes, the loss of scales occurred several times during the evolution of this group. Interestingly, we found that the presence/absence of scales bear virtually no phylogenetic signal indicating that a scaleless state arose independently in distant fish orders, as for instance in the Siluriformes or in the Acipenseriformes (Fig. 1 and 2 for the 304 and 11,638 species datasets, respectively).

Interestingly, our results based on both datasets revealed a tight association between the scaleless state and a benthic way of life. However, the strong correlation we revealed does not indicate whether the scaleless phenotype is a cause or a consequence of a benthic habitat preference. If scale loss were a consequence of a benthic ecology, then we would expect virtually no open water species displaying a scaleless integument. To the contrary, if a benthic habitat preference were a consequence of scale loss in ancestors with open water habitat preference, then we would expect at least some scaleless taxa in open waters, as the loss of scales would initially occur there, before a translocation into the benthic habitat. After a careful examination of our table of the scaling status and the habitat preference, it appears that 19 scaleless fish families live in open waters Table (S4). We can mention, for instance, the Stomiidae (a deep-sea fish family comprising 287 species, of which 41 are present in the 11,638 species dataset), the Salangidae (a family of icefishes with 17 out of the 20 species

represented in the 11,638 species dataset), the enigmatic family Regalecidae (with 1 and 2 out of the 3 species represented in our reduced and large datasets, respectively), and the Galaxiidae (even though some benthic species are comprised among the 53 species of this family, of which 27 and 2 are included in the large and the reduced datasets, respectively). The fact that scaleless groups live in open waters supports the hypothesis that scale loss came first, as a likely pre-adaptation to colonize the benthic environment.

While the scaleless phenotype is found in strong association with the benthic habitat and likely leads to a benthic way of life in ray-finned fishes, the biological meaning of this association is difficult to assert. Some potential explanations could rely in the increased cutaneous respiration in scaleless species (Park and Kom 1999; Park 2002) in a benthic environment characterized by a reduced oxygen content and limited water flow, as compared to open water environments. Another advantage could rely in the increased immune response of scaleless skin (Holm et al. 2017) when confronted to a microbial-rich benthic environment. We could thus argue that fishes having lost their scales are better adapted to the benthic environment, facilitating the colonization of this niche. Because the association between a scaleless phenotype and a benthic way of life has evolved repeatedly and independently many times, these parallel evolutionary trajectories suggest that a scaleless integument has strong selective value in the benthic environment.

### REGAINING SCALES IS UNLIKELY

In our analyses, we inferred very few instances of scale re-acquisition after scale loss events in ray-finned fishes, making the case that scale losses are hardly reversible. The few scale re-acquisition events were inferred either with relatively low probability (e.g., Opisthognathidae family), questioning their validity, or in families with poorly resolved phylogenetic relationships. For instance, in the 304 species phylogeny, we inferred a scale re-acquisition within the Anguilliformes order, more specifically in the Anguillidae family (Fig. S1, Fig. S2 and Fig. S3). However, because the phylogenetic relationships within the Anguilliformes is still debated (e.g. different resolutions of the branching order in the phylogenies of Johnson et al. 2012 and Santini et al. 2013), alternative branching patterns may cancel the inference of a scale re-acquisition. When using the Rabosky et al. (2018) phylogeny, scale gains were inferred in relatively enigmatic taxa. The position of such taxa within this large phylogeny may still lack resolution. Indeed, when examining other published lineage-specific phylogenies for the groups in which suspicious scale re-acquisition was inferred, we can observe that the relationships are often different from the ones found in the large phylogeny proposed by Rabosky et al. (2018). As a matter of fact, specific phylogenies of *Clariger* and *Luciogobius* genera (Yamada et al.

2009), *Parupeneus* (Song et al. 2014), *Lycodes* (Turanov et al. 2017), *Cryptacanthodes* (Radchenko et al. 2011), *Ocosia* (Smith et al. 2018), *Notothenia* (Near et al. 2018), *Lophiocharon* (Arnold and Pietsch 2012), *Perulibatrachus* (Rice and Bass 2009), and *Stomias* (Kenaley et al. 2014) genera all present differences with the topology of Rabosky et al. (2018) we used in our reconstruction (the problematic subtrees are presented Fig. S11). Consequently, at least some scale re-acquisitions could be artifacts resulting from topological errors in the phylogeny of Rabosky et al. (2018).

In any case, we here show that scale loss events occurred multiple times along the evolutionary history of actinopterygians, while scale re-acquisitions were extremely rare or non-existent. These findings suggest that the gene regulatory network underlying scale formation is difficult to reassemble after it has been dismantled during a scale loss event.

Recent studies showed that the absence of scales in ray-finned fishes may be associated with genetic changes (Liu et al. 2016). For instance, in the secretory calcium-binding phosphoprotein (SCPP) gene family, which is important for scale mineralization in various ray-finned fishes (Liu et al. 2016; Lv et al. 2017), the SCCP1 and SCCP5 genes have been proposed as candidates genes linked to scale presence or absence (Liu et al. 2016). Yet, while these genes are linked to the scaleless phenotypes in some species (e.g., *Ictalurus punctatus*, *Electrophorus electricus*; Liu et al. 2016), other scale losses could not be linked to these specific genes (e.g., *Sinocyclocheilus anshuensis*; Lv et al. 2017). As such, different genes and/or set of genes may be underlying the presence or absence of scales (Lv et al. 2017) and more research is needed to uncover upstream genetic switches.

### TRUNK BONY PLATES EMERGE ON A SCALELESS INTEGUMENT

The emergence of TBP occurred in several places of the studied phylogenies (Fig. 1 and 2 for the 304 and 11,638 species datasets, respectively). The presence of TBP structures is found in different unrelated taxa, and thus bears virtually no phylogenetic signal (Table 1). We here show that there is a common ground needed for the emergence of such plates on the trunk of ray-finned fishes, which is the absence of scales. The functional relations between these two traits remain however uncertain. Interestingly, it also appears that the acquisition of TBP is hardly reversible in the 304 species phylogeny, yet possible but extremely rare in the 11,638 one. This discrepancy can be explained by the fact that the few clades showing TBP losses in the 11,638 species phylogeny are not present in the 304 species dataset (Auchenipteridae, Harpagiferidae, Tetraogidae), while some clades are present, yet represented with only few species (e.g., Cottidae, Cyclopteridae, Nototheniidae). Interestingly, however, most of the TBP losses

inferred using the 11,638 species phylogeny occurred in groups in which internal phylogeny is not perfectly resolved. The large phylogeny of Rabosky et al. (2018) is indeed locally different from other published specialized phylogenies focusing, for instance, on the Siluriformes order (Sullivan et al. 2006), the Tetraogidae family (Smith et al. 2018) and the Nototheniidae and Harpagiferidae families (Near et al. 2018).

The discovery that TBP emerged on taxa with scaleless integuments together with the strong association between scaleless integument and benthic habitat preference explains the observation that almost all extant fishes displaying TBP have a benthic habitat preference. One main function of scales is the physical protection they provide (Vernerey and Barthelat 2014). It is thus possible that, in the absence of scales, and given the suggested complexity of the genetic control of scale development, simpler alternative developmental pathways can be reached leading to the emergence of a different protective integument in the form of bony plates on the trunk. The numerous independent acquisitions of a hard armor composed of TBP are indicative of the reduced genetic complexity underlying their emergence given the actinopterygian genetic background. Furthermore, once TBP have been acquired, the low rate of secondary losses indicates that they likely confer some evolutionary advantages, as previously suggested by Vickaryous and Sire 2009.

### LIMITATIONS OF THE STUDY

Our results give new insight into the interconnected evolution among different integument structures in ray-finned fish. However, different elements could limit the outcome and interpretation of our study.

First, investigating the evolution of traits that are still debated within the scientific community is a challenging task. Indeed, clear consensus about the distinction between different integument structures in fish has yet to be reached, and we thus opted for a macro-structural approach differentiating micrometric scale-like elements from macromeric bony plate-like elements. More research is needed to understand the homology among these categories of structures, in particular through paleontological and developmental genetics studies.

Second, we have mentioned some situations that may hamper the complete resolution, and with high confidence, of the ancestral state reconstructions. Errors in the phylogenetic tree and lack of resolution in parts of the tree may mislead the ancestral state reconstructions. Indeed, the robustness of the phylogeny is paramount for proper reconstruction of ancestral states. For instance, the discrepancies between the relationships within the Siluriformes order in the large phylogeny of Rabosky et al. (2018), as compared to the lineage-specific Siluriformes phylogeny of Sullivan et al. (2006) may explain the loss of TPB we inferred in some Siluriformes taxa. We have also observed that the



impressive taxonomic sampling yet coupled with a reduced amount of sequence data characterizing the phylogenetic tree of Rabosky et al. (2018) resulted in some differences when analyzing it with the two ancestral state reconstruction methods (i.e., stochastic mapping vs Bayestrait). To the contrary, the more robust phylogeny of Hughes et al. (2018) but with a reduced taxonomic sampling showed virtually no disparity between the results obtained with the same two methods. In the phylogeny of Rabosky et al. (2018), we pointed out controversial phylogenetic relationships within several genera, that is, at a recent phylogenetic scale were high-quality sequences of fast-evolving markers are required for a fine resolution. For example, some well-recognized families and genera were found to be polyphyletic, a problem that most likely explains the few unexpected recent TBP loss events.

Third, the lack of precise morphological knowledge about the traits of interest in some poorly described taxa can lead to erroneous trait attributions, and thus to some artifactual reconstructions (mistaken gains or loss of structures). This situation might be found in the Gobiidae family with the genus *Luciogobius*, in the family Cottidae with the genus *Clinocottus*, or in the family Tetraogoniidae with the genus *Ocosia*, among others.

Despite the above-mentioned limitations explaining why our study cannot certify the accuracy of every single reconstructed event along with the evolution of the integument structures in ray-finned fishes, the general patterns we present are robust to changes in analytical methods, dataset size, and phylogenetic uncertainty. Overall, the tested conditions yielded very similar results supporting our conclusions.

## Conclusion

We here demonstrate that scale loss events occurred several times, in an independent manner along the evolution of Actinopterygii, the most species-rich group of vertebrates. We observe that these scale losses are hardly reversible as scale re-acquisition is extremely unlikely. We show that the scaleless phenotype is associated to a benthic habitat preference, and we argue that following a scale loss event, fishes tend to colonize the floors of oceans and water bodies, adopting a benthic lifestyle. The repeated and parallel colonization of the seafloor after a scale loss event indicates that the scaleless phenotype most probably confers a selective advantage in this particular habitat. We also show that the multiple emergences of TBP are phylogenetically independent. We demonstrate that their emergence is dependent over a previous scale loss event. Indeed, these TBP are never present in scaled bodies and thus only arise in fish displaying a scaleless integument, in a “gain after scale loss” evolutionary sequence. The precise mechanisms ruling the interplay between the loss of

scales and the emergence of TBP remain however to be studied further. Studies focusing on the gene regulatory networks implicated in the transition between integument structures along evolution could shed light upon the transition between scales and TBP in ray-finned fishes. Finally, our findings support the hypothesis that trunk integument structures are functional innovations that contributed to the radiation of ray-finned fishes in the aquatic environment.

## ACKNOWLEDGMENTS

We thank the University of Geneva BioSC service and Dr. José Manuel Nunes for advices on the statistical tests. We thank Luigi Manuelli and Killian Perrelet for comments on the manuscript and Slim Chraïti for his photographic work. The computations were performed at University of Geneva on the Baobab cluster. This research was supported by the Swiss National Science Foundation (project N° 310030\_185327 to JIMB), by the department GENEV, and by the Claraz Donation. The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

A.-L. gathered the data and performed the analyses. J.I.M.-B. designed and supervised the study. Both authors analyzed and discussed the results, wrote and edited the manuscript and approved its submission.

## DATA ARCHIVING

Supporting Information is available in the Supporting Information section. Supplementary material will be available upon acceptance on dryad accession number <https://doi.org/10.5061/dryad.cfxpvnv4r>.

## LITERATURE CITED

- Aberer, A. J., K. Kobert, and A. Stamatakis 2014. Exabayes: massively parallel bayesian tree inference for the whole-genome era. *Mol. Biol. Evol.* 31:2553–2556.
- Alfaro, M. E. 2018. Resolving the ray-finned fish tree of life. *Proc. Natl. Acad. Sci. U. S. A.* 115:6107–6109.
- Arnold, R. J. and T. W. Pietsch 2012. Evolutionary history of frogfishes (Teleostei: lophiiformes: antennariidae): a molecular approach. *Mol. Phylogenet. E* 62:117–129. Elsevier Inc.
- Benton, M.. 2004. *Vertebrate Paleontology*, 3rd ed. Blackwell Science Ltd.
- Covain, R., S. Fisch-Muller, C. Oliveira, J. H. Mol, J. I. Montoya-Burgos, and S. Dray 2016. Molecular phylogeny of the highly diversified catfish sub-family Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. *Mol. Phylogenet. E* 94:492–517.
- Johnson, D., G., Ida, H., Sakaue, J., Sado, T., Asahida, and M. Miya 2012. A “living fossil” eel (Anguilliformes: protanguillidae, fam. nov.) from an undersea cave in Palau. *Proc. R. Soc. B Biol. Sci.* 279:934–943.
- Friedman, M. and M. D. Brazeau 2010. A reappraisal of the origin and basal radiation of the Osteichthyes. *J. Vertebr. Paleontol.* 30:36–56.
- Fritz, S. A. and A. Purvis 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conserv. Biol.* 24:1042–1051.
- Froese, R. and D. Pauly 2011. Fishbase. [www.fishbase.org](http://www.fishbase.org)
- Gemballa, S. and P. Bartsch 2002. Architecture of the Integument in Lower Teleostomes: functional Morphology and Evolutionary Implications. *J. Morph.* 309:290–309.
- Holm, H. J., S. Skugor, A. K. Bjelland, S. Radunovic, S. Wadsworth, E. O. Koppang, et al. 2017. Contrasting expression of immune genes in scaled

- and scaleless skin of Atlantic salmon infected with young stages of *Lepoepitheirus salmonis*. *Dev. Comp. Immunol.* 67:153–165. Elsevier Ltd.
- Huelskenbeck, J. P., R. Nielsen, and J. P. Bollback 2003. Stochastic mapping of morphological characters. *Syst. Biol.* 52:131–158.
- Hughes, L. C., G. Ortí, Y. Huang, Y. Sun, C. C. Baldwin, A. W. Thompson, et al. 2018. Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. *Proc. Natl. Acad. Sci. U. S. A.* 115:6249–6254.
- Ichiro, S., I. Mikio, Y. Hiroyuki, and M. Masato 2013. Teeth and ganoid scales in *Polypterus* and *Lepisosteus*, the basic actinopterygian fish: an approach to understand the origin of the tooth enamel. *J. Oral Biosci.* 55:76–84. Elsevier.
- Keating, J. N. and P. C. J. Donoghue 2016. Histology and affinity of anaspids, and the early evolution of the vertebrate dermal skeleton. *Proc. R. Soc. B Biol. Sci.* 283. 20152917
- Kenaley, C. P., S. C. Devaney, and T. T. Fjeran 2014. The complex evolutionary history of seeing red: molecular phylogeny and the evolution of an adaptive visual system in deep-sea dragonfishes (stomiiformes: stomiidae). *Evolution (N. Y.)*. 68:996–1013.
- Lees, J., T. Märss, M. V. H. Wilson, T. Saat, and H. Špilev 2012. The sculpture and morphology of postcranial dermal armor plates and associated bones in gasterosteiforms and syngnathiforms inhabiting Estonian coastal waters. *Acta Zool* 93:422–435.
- Liu, Z., S. Liu, J. Yao, L. Bao, J. Zhang, Y. Li, et al. 2016. The channel catfish genome sequence provides insights into the evolution of scale formation in teleosts. *Nat. Commun.* 7: 11757.
- Lv, Y., K. Kawasaki, J. Li, Y. Li, C. Bian, Y. Huang, et al. 2017. A genomic survey of SSCP family genes in fishes provides novel insights into the evolution of fish scales. *Int. J. Mol. Sci.* 18:1–11.
- Meunier, F. J. and P. M. Brito 2004. Histology and morphology of the scales in some extinct and extant teleosts. *Cybium* 28:225–235.
- Mongera, A. and C. Nüsslein-Volhard 2013. Scales of fish arise from mesoderm. *Curr. Biol.* 23:R338–R339. Elsevier.
- Moyle, P. B. and J. J. Cech 2004. *Fishes, An introduction to Ichthyology*, 5th ed. (B. Cummings, ed).
- Near, T. J., D. J. MacGuigan, E. Parker, C. D. Struthers, C. D. Jones, and A. Dornburg 2018. Phylogenetic analysis of Antarctic notothenioids illuminates the utility of RADseq for resolving Cenozoic adaptive radiations. *Mol. Phylogenet. E* 129:268–279. Elsevier.
- Nelson, J. S., T. C. Grande and M. V. H. Wilson 2016. *Fishes of the World*, 5th ed. Wiley.
- Orme, C. D. L., R. P. Freckleton, G. H. Thomas, T. Petzoldt, and S. A. Fritz 2013. The caper package: comparative analyses of phylogenetics and evolution in R. <http://caper.r-forge.r-project.org>. *Google Sch.*
- Pagel, M., A. Meade and D. Barker, 2004. Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* 53:673–684.
- Paradis, E., J. Claude, and K. Strimmer 2004. APE: analyses of Phylogenetics and Evolution in R language. *Bioinformatics* 20:289–290.
- Park, J. Y. 2002. Structure of the skin of an air-breathing mudskipper, *Periophthalmus magnuspinnatus*. *J. Fish Biol.* 60:1543–1550.
- Park, J. Y. and I. - S. Kom 1999. Structure and histochemistry of skin of mud loach *Misgurnus anguillicaudatus* (Pisces, Cobitidae), from Korea. *Korean J. ichthyology* 11:109–116.
- Porter, M. M., E. Novitskaya, A. B. Castro-Ceseña, M. A. Meyers, and J. McKittrick 2013. Highly deformable bones: unusual deformation mechanisms of seahorse armor. *Acta Biomater* 9:6763–6770. Acta Materialia Inc.
- Qu, Q., M. Zhu, and W. Wang 2013. Scales and Dermal Skeletal Histology of an Early Bony Fish *Psarolepis romeri* and Their Bearing on the Evolution of Rhombic Scales and Hard Tissues. *PLoS One* 8: e61485
- Rabosky, D. L., J. Chang, P. O. Title, P. F. Cowman, L. Sallan, M. Friedman, et al. 2018. An inverse latitudinal gradient in speciation rate for marine fishes. *Nature* 559:392–395. Springer US.
- Radchenko, O. A., I. A. Chereshev, A. V. Petrovskaya, and D. V. Antonenko 2011. Relationships and position of wrymouths of the family cryptacanthodidae in the system of the suborder Zoarcoidei (Pisces, Perciformes). *J. Ichthyol.* 51:487–499.
- Rambaut, A., A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* 67:901–904.
- Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. E* 3:217–223.
- Rice, A. N. and A. H. Bass 2009. Novel vocal repertoire and paired swimbladders of the three-spined toadfish, *Batrachomoeus trispinosus*: insights into the diversity of the Batrachoididae. *J. Exp. Biol.* 212:1377–1391.
- Rivera-Rivera, C. J. and J. I. Montoya-Burgos 2017. Trunk dental tissue evolved independently from underlying dermal bony plates but is associated with surface bones in living odontode-bearing catfish. *Proc. R. Soc. B Biol. Sci.* 284: 20171831.
- Santini, F., X. Kong, L. Sorenson, G. Carnevale, R. S. Mehta, and M. E. Alfaro 2013. A multi-locus molecular timescale for the origin and diversification of eels (Order: anguilliformes). *Mol. Phylogenet. E* 69:884–894. Elsevier Inc.
- Schultze, H. P. 2018. Hard tissues in fish evolution: history and current issues. *Cybium* 42:29–39.
- Sire, J.-Y. 1993. Development and fine structures of the bony scutes in *Corydoras arcuatus* (Siluriformes, Callichthyidae). *J. Morphol.* 215:225–244.
- Sire, J.-Y., F. Allizard, O. Babiari, J. Bourguignon, and A. Quilhac 1997. Scale development in zebrafish (*Danio rerio*). *J. Anat.* 190:545–561.
- Sire, J. Y., P. C. J. Donoghue, and M. K. Vickaryous 2009. Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. *J. Anat.* 214:409–440.
- Sire, J. Y. and A. Huisseune 2003. Formation of dermal skeletal and dental tissues in fish: a comparative and evolutionary approach. *Biol. Rev. Camb. Philos. Soc.* 78:219–249.
- Smith, W. L., E. Everman, and C. Richardson 2018. Phylogeny and Taxonomy of Flatheads, Scorpionfishes, Sea Robins, and Stonefishes (Percomorpha: scorpaeniformes) and the Evolution of the Lachrymal Saber. *Copeia* 106:94–119.
- Song, H. Y., K. Mabuchi, T. P. Satoh, J. A. Moore, Y. Yamanoue, M. Miya, et al. 2014. Mitogenomic circumscription of a novel percomorph fish clade mainly comprising “Syngnathoidei” (Teleostei). *Gene* 542:146–155. Elsevier B.V.
- Stöver, B. C. and K. F. Müller 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* 11:1–9.
- Turanov, S. V., Y. P. Kartavtsev, Y. H. Lee and D. Jeong 2017. Molecular phylogenetic reconstruction and taxonomic investigation of eelpouts (Cottoidei: zoarcales) based on Co-1 and Cyt-b mitochondrial genes. *Mitochondrial DNA Part A DNA Mapping, Seq. Anal.* 28: 547–557.
- Vernerey, F. J. and F. Barthelat 2014. Skin and scales of teleost fish: simple structure but high performance and multiple functions. *J. Mech. Phys. Solids* 68:66–76. Elsevier.
- Vickaryous, M. K. and J. Y. Sire 2009. The integumentary skeleton of tetrapods: origin, evolution, and development. *J. Anat.* 214:441–464.
- Wainwright, P. C. and S. J. Longo 2017. Functional Innovations and the Conquest of the Oceans by Acanthomorph Fishes. *Curr. Biol.* 27:R550–R557.

Yamada, T., T. Sugiyama, N. Tamaki, A. Kawakita, and M. Kato 2009. Adaptive radiation of gobies in the interstitial habitats of gravel beaches accompanied by body elongation and excessive vertebral segmentation. *BMC Evol. Biol.* 9:1–14.

Zhu, D., C. F. Ortega, R. Motamedi, L. Szewciw, F. Vernerey, and F. Barthelat 2012. Structure and mechanical performance of a “modern” fish scale. *Adv. Eng. Mater.* 14:185–194.

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Reconstruction through stochastic mapping of the scale / scaleless trait evolutionary history on a 304 species phylogeny presented by Hughes et al (2018).

Fig. S2 Reconstruction of the scale / scaleless trait evolutionary history on a 304 species phylogeny presented by Hughes et al (2018).

Fig. S3 Reconstruction of the scale / scaleless trait evolutionary history, accounting for phylogenetic uncertainty, on the 304 species phylogeny presented by Hughes et al (2018).

Fig. S4 Reconstruction through stochastic mapping of the scale / scaleless trait evolutionary history on the 11,638 species phylogeny presented by Rabosky et al (2018).

Fig. S5 Reconstruction of the scale / scaleless trait evolutionary history on the 11638 species phylogeny presented by Rabosky et al (2018).

Fig. S6 Reconstruction through stochastic mapping of the presence/absence of trunk plates trait evolutionary history on the 304 species phylogeny presented by Hughes et al. (2018).

Fig. S7 Reconstruction of the presence/absence of trunk bony plates (TBP) evolutionary history on the 304 species phylogeny presented by Hughes et al (2018).

Fig. S8 Reconstruction of the presence/absence of trunk bony plates (TBP) evolutionary history, accounting for phylogenetic uncertainty, on the 304 species phylogeny presented by Hughes et al (2018).

Fig S9. Reconstruction through stochastic mapping of the presence/absence of trunk bony plates (TBP) evolutionary history on the 11638 species phylogeny presented by Rabosky et al (2018).

Fig. S10 Reconstruction of the the presence/absence of trunk bony plates (TBP) evolutionary history on the 11,638 species phylogeny presented by Rabosky et al (2018).

Fig S.11 Ancestral state reconstruction of the presence / absence of scales in actinopterygians indicates that scale reacquisition is rare and should be considered with caution.

Table S1. List of 304 species of Actinopterygii and their classification for three characters.

Table S2 List of 11,638 species of Actinopterygii from the dataset in Rabosky et al. (2018) and their classification for three characters.

Table S3 Model selection for the two datasets we used, the 304 species of Hughes et al. (2018) and the 11,638 species of Rabosky et al. (2018) and for two different binary traits, presence / absence of scales and presence / absence of trunk bony plates (TBP).

Table S4. Number of scaleless species living in open waters, which are included in the two datasets we analyzed: Hughes et al. (2018) and Rabosky et al. (2018).

Table S2. List of the 11'638 species of Actinopterygii from the dataset in Rabosky et al. (2018) and their classification for three characters.

Supplementary Materials

Supplementary Materials