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Interspecific differences and ecological correlations between scale number and skin structure in freshwater fishes

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Abstract

Fish skin is mainly composed of the epidermis, dermis, and its derivative scales. There is a wide diversity in scale number in fishes, but the diversity of skin structure lacks systematic histological comparison. This research aimed to improve our understanding of the functional relationship between the scale number and the skin structure in freshwater fishes and to determine which ecological factors affect the scale number and skin structure. First, we presented a method to quantify skin structure in fish and histologically quantified the skin structure of 54 freshwater fishes. Second, we collected the scale number and habitat information of 509 Cyprinidae fishes in China and explored which ecological factors were related to their scale number. Third, common carp and scaleless carp were used as models to study the effects of scale loss on swimming. We found a strong negative correlation between scale thickness and scale number. The main factor affecting the skin structure of fishes was the species' water column position, and the skin of benthic fishes was the most well-developed (thicker skin layers (dermis, epidermis) or more/larger goblet cells and club cells). The scale number was related to two factors, namely, temperature and water column position, and cold, benthic and pelagic adaptation may have contributed to increased scale numbers. Only in benthic fishes, the more well-developed their skin, the more scales. In common carp, scale loss did not affect its swimming performance. In summary, we suggest that there is a rich diversity of skin structure in freshwater fishes, and the scales of fish with well-developed skin tend to degenerate (greater number/smaller size/thinner, or even disappear), but the skin of fish with degenerated scales is not necessarily well developed.

Key words: ecological adaptation, functional antagonism, scale degeneration, scale number, skin structure.

Scales are widespread elements of the postcranial skeleton or the integumentary skeleton (Sire et al. 2009). They play an important role in mechanical protection (Torres et al. 2008), acting as armor to protect fish from piscivorous predators and scrapes from rocks and branches. Fish scales can resist piscivorous predators' bites to a certain extent (Meyers et al. 2012; Zhu et al. 2013; Yang et al. 2014), but most piscivorous predators tend to swallow their prey whole (Mcgee et al. 2015; Gu et al. 2021), which indicates that the resistance to piscivorous predators is a secondary role of the scales. Another important role of scales is their resistance to pathogenic microorganisms. For example, fish with no scales or fine scales are more susceptible to white spot disease *Ichthyophthirius multifiliis* in their breeding environment (Sigh et al. 2004; Wang and Dickerson 2002; Mallik et al. 2015). Similarly, fully scaled carp were more resistant to white spot disease than scaleless carp, linear mirror carp, and scattered mirror carp (Price and Clayton 1999).

The skin or integument forms the external covering of the body and performs a number of important functions in fishes.

It protects the body from injury and infections and helps with respiratory, excretory, and osmoregulatory functions (Elliott 2000). Fish skin is made up of two layers with scales between them, the epidermis and dermis, both of which have protective functions. The epidermis mainly consists of goblet cells (mucous cells), club cells (alarm substance cells), and several layers of flattened stratified epithelial cells. Epithelial cells mainly have protective and supportive functions and certain functions of repair, mucus secretion, ion exchange, etc. (Elliott 2000). The main function of goblet cells is to secrete mucus, which has lubricating and protective functions, followed by other functions, such as repair and immunity (Elliott 2000). The contents of club cells cannot be released voluntarily; they are released only when the cell ruptures. The release of chemical alarm cues is a reliable alert to other individuals that an actively foraging predator is present (Chivers et al. 2007). Furthermore, in some fish, club cells also have other functions, such as the repair and secretion of mucus (Elliott 2000; Xie 2009).

Mucous cells and club cells are the most common derivative of the epidermis, so we considered quantifiable skin structure mainly to include the thickness of the epidermis, scales and dermis, and the size and number of mucous cells and club cells (in subsequent descriptions, skin does not include scales). Like scales, skin (epidermis and dermis) also has the function of mechanical protection, and mucus and other substances secreted by mucous cells and club cells also have the function of resisting pathogens and reducing friction with the substrate (Agrawal et al. 1992; Rai et al. 2012; Pandey et al. 2021). Scales and skin both have protective functions, so their development may be mutually antagonistic. Specifically, we hypothesize that it is impossible for the same species of fish to both have well-developed scales (larger size or less number) and well-developed skin (thicker skin layers (dermis, epidermis) or more/larger goblet cells and club cells), so fish with well-developed scales will have less mucus and thinner skin. However, due to the lack of systematic methods to quantify skin structure, the description above is only a hypothesis, and there is no research on the relationship between the skin structure and scale number of interspecific fishes.

On the other hand, there is a wide diversity in scale number, size (relative size, compared to body size), and shape in fish, which makes the scales one of the most important indicators in the morphological taxonomy of fishes. Current studies mainly focus on the evolution of fish scale shape (Viertler et al. 2021; Grady et al. 2022; Wainwright et al. 2022), but there are few studies on the evolution of number and size. Existing research reports that there are three main aspects causing fish scales to degrade (greater number/smaller size/thinner) or even disappear. The first aspect is related to an adaptation to benthic or burrowing life, for example, in loach, catfish, and eel. These fish are scaleless or have fine scales, making their bodies supple and easily bent (Ghosh et al. 2014; Szewciw et al. 2017; Seo et al. 2020) for benthic or burrowing life. The second aspect is related to an adaptation to low temperature. It has long been known that fish from low latitudes usually have fewer scales than those of the same species from high latitudes, and it has been assumed that this decrease in the number of scales is due to the higher temperature under which the individuals develop at low latitudes (Mottley 1931). Specifically, under low-temperature conditions, development (the formation and differentiation of organs) is slower than growth (individual size increases) and scales appear later when the individual is larger. Since the initial size of the scales is conservative, a larger individual will have more scales covering its body. Conversely, under high-temperature conditions, scales appear earlier, and when individuals are smaller, this causes them to have fewer scales covering them (Hubbs 1926; Levin 2010; Levin 2011). The increase in the number of vertebrae of fish in cold regions has the same mechanism (Hubbs 1922; Bolotovskiy and Levin 2018), so the number of scales and vertebrae in fish tends to be correlated (Chen 1998; Yue 2000). The third aspect is related to gene mutation or deletion, which usually result in complete loss or irregularity of scales, as found in scaleless carp and mirror carp *Cyprinus carpio*. For example, mutations in the fibroblast growth factor receptor 1 (*Fgfr1*) gene or the ectodysplasin (*Eda*) gene cause the scaleless phenotype (Harris et al. 2008; Rohner et al. 2009; Zhou et al. 2018; Kim et al. 2019). However, the three aspects above cannot explain the patterns in all fishes with numerous scales, such as silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*, which means that there are other factors that affect the number of fish scales.

In summary, this study raised three main scientific questions: Are there other factors that affect fish scale number? What ecological factors cause differences in fish skin structure? What is the relationship between scale number and skin structure in fish?

In this study, tissue sections and AB-PAS staining were performed on the lateral skin of 55 species of bony freshwater fishes (including 33 species of Cyprinidae fishes, 21 species of non-Cyprinidae fishes and scaleless carp), and the relationship between their scale number and skin structure was compared. In addition, common carp and scaleless carp were used as research models to explore the effects of scale loss on swimming performance. Furthermore, we collected data on the ecological factors of the habitat and the scale numbers of 509 species of Cyprinidae fishes in China to explore which ecological factors are related to scale number.

Materials and Methods

The degree of scale degradation (DSD) formula

The smaller the fish scales are, the greater their number (the thinner the scales are, refer to the results below), and the more degraded they are. In this study, the degree of scale degradation (DSD) was used to describe and quantify the number of scales. To facilitate data collection and simplify the quantification process, we used the following formula to calculate the DSD of different fishes:

$$\text{DSD} = \sqrt{\text{SOLL} \times (\text{SALL} + \text{SBLL})} \quad \text{or} \quad \sqrt{\text{TS} \times \text{LS}}$$

where SOLL represents the number of scales on the lateral line, SALL represents the number of scales above the lateral line (the number of scale rows between the origin of the dorsal fin and lateral line), SBLL represents the number of scales below the lateral line (the number of scale rows between the origin of the pelvic fin and lateral line), TS represents transverse scales (the number of scales from the upper angle of the gill aperture to the last scale at the base of the caudal fin along the lateral axis), and LS represents longitudinal scales (the number of scales sloping downwards from the dorsal fin to the midline of the abdomen). TS and LS are usually used in fish with no lateral lines or with incomplete lateral lines (Chen 1998; Yue 2000). For details, please refer to Figure 1A.

To assign a value to the DSD of the scaleless fishes, we refer to the DSD of other fishes. In general, the DSD of fishes with visible scales will not exceed 80 (refer to the following results). According to the DSD distribution of other fish, we define the DSD of scaleless fishes as 100. Coincidentally, it can be simply understood as 100% degradation of scaleless fishes. A few fishes have very small scales that can only be seen in magnification, their calculated DSD exceeds 100, but considering that the DSD of scaleless fish is 100, we also define their DSD as 100.

Experimental animals and histology

There were 33 species of Cyprinidae fishes from 31 genera, and 21 species of non-Cyprinidae fishes, including 15 families and 20 genera. For specific information, please refer to Supplementary Table S1. The experimental fish were mainly collected in the wild, and a small amount was purchased in the aquarium market, they were domesticated for a short time in our laboratory.
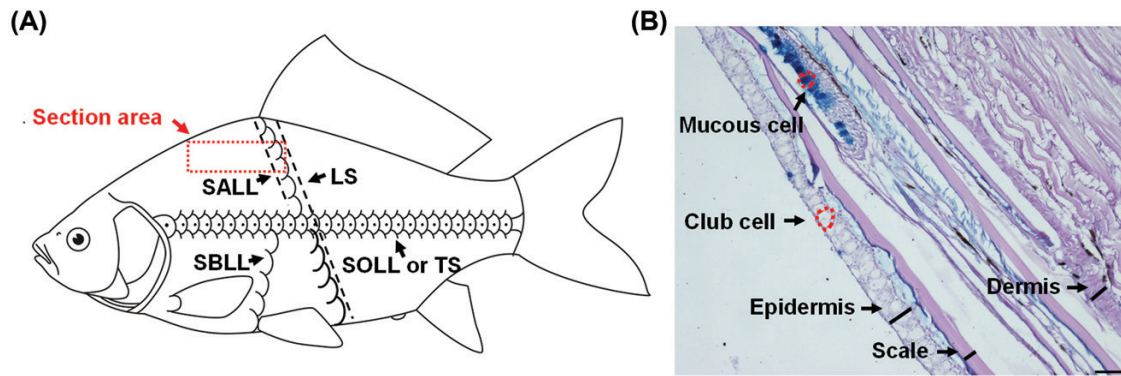
Figure 1 Reference figure for quantitative indicators. (A) Reference figure for quantitative scale number. The red rectangular box represents the skin tissue section area. (B) Reference figure for quantitative skin structure. The scale is 100 µm. SOLL: the number of scales on the lateral line; SALL: the number of scales above the lateral line; SBLL: the number of scales below the lateral line; TS: transverse scales; LS: longitudinal scales. TS and LS are usually used in fish with no lateral lines or with incomplete lateral lines.

Three fish of each species used for histological observation were kept under the same breeding conditions at 20 °C for 3 days, and then euthanized with an overdose of MS-222 (tricaine methane sulfonate) and their indicators related to the number of scales were counted, including SOLL (the number of scales on the lateral line), SALL (the number of scales above the lateral line), SBLL (the number of scales below the lateral line), TS (transverse scales) and LS (longitudinal scales). Next, their lateral skin tissue (above the lateral line, and before the dorsal fin) (Figure 1A) was collected, fixed in 4% paraformaldehyde for approximately 4 h at room temperature and then transferred to a 70% alcohol solution for long-term preservation. Although there were scales in the tissues, the scales of juvenile fish were not well developed, so we did not decalcify the tissues to make the scales softer. Traditional paraffin sections (6 µm) were stained with an AB-PAS staining kit (DG1014-6 × 100ML, Dingguo). All sections were observed using a microscope (Nikon SMZ25, Japan) and photographed (100×). Three complete skin tissues of each section were randomly selected for photographing. In total, we quantified 9 images for each species. Figures of individual, scale, and skin histology of all experimental fish are available in Supplementary File 2.

Each image was quantified using ImageJ software (National Institute of Health, Bethesda, MD). We sequentially quantified the thickness of the epidermis (outermost layer), scales, and dermis in the picture (a representative area of intermediate thickness selected). Next, 10 mucous cells and 10 club cells were randomly selected to calculate their mean cross-sectional area. Then, we quantified the length of the skin tissue in the image and counted the number of mucous cells and club cells to calculate their numbers in a 1 mm cross-section of skin tissue (Figure 1B). Finally, we calculated these indicators for each image, including the relative epidermis thickness (RET), relative scale thickness (RST), relative dermal thickness (RDT), relative epidermis and dermal thickness (REDT), relative total area of mucous cells (RTAMC), relative total area of club cells (RTACC), and relative total area of mucous and club cells (RTAMCC), using the following formulas:

$$RET = ET/BW$$

$$RST = ST/BW$$

$$RDT = DT/BW$$

$$REDT = RET + RDT$$

$$RTAMC = MA_{MC} \times N_{MC}/BW^2$$

$$RTACC = MA_{CC} \times N_{CC}/BW^2$$

$$RTAMCC = RTAMC + RTACC$$

where ET represents the epidermis thickness, ST represents the scale thickness, DT represents the dermal thickness, and BW represents the body width (the maximum width of the body, except the head and fin ray). These indicators are divided by body width to eliminate the effect of fish size (the body width is in the same plane as the cut plane, so we use it instead of the body length). MA_{MC}/MA_{CC} represents the mean cross-sectional area of mucous/club cells, and N_{MC}/N_{CC} represents the number of mucous cells/club cells in a 1 mm cross-section of skin. These indicators are divided by body width squared to eliminate the effect of fish size (considering that both RTAMC and RTACC are area data divided by body width squared to eliminate units).

To obtain an overall indicator of skin structure, we standardized the quantitative data of REDT and RTAMCC for each image of all the species using a Z-score in IBM SPSS Statistics (version 21.0, Armonk, New York, USA). We calculated the indicator of the comprehensive skin index (CSI) by the following formula:

$$CSI = Z(REDT) + Z(RTAMCC)$$

Where Z(REDT) represents the Z-score (REDT) of each image relative to all data and Z(RTAMCC) represents the same for RTAMCC.

The CSI can reflect the protective function of the skin, with a higher value indicating greater protective function, that is, more and larger mucous cells and club cells or thicker skin or both.

The data measured for each experimental fish can be found in Supplementary Table S2.

Phylogenetic analysis

We downloaded all the DNA sequences of all of the experimental fishes above that contain the 13 protein-coding genes of the complete mitochondrial genome and then aligned the sequence with the MAFFT Online Service (Katoh et al. 2019).

All sequences were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/nuccore/), and GenBank accession numbers are provided in Supplementary Table S3. The 13 protein-coding genes were partitioned by the first, second, and third codons in PhyloSuite v1.2.1 (Zhang et al. 2020). Finally, we constructed a maximum likelihood phylogenetic tree using the default setting of IQ-TREE (Trifinopoulos et al. 2016), and the best model is shown in Supplementary Table S4. We referred to previous studies (Ding 1994; Chen 1998; Yue 2000; Guo et al. 2021) and our laboratory's long-term field investigation experience to classify the water column position of each experimental fish. In this study, the water column position can be divided into benthic (those primarily living on the substrate), sub-benthic (those primarily living lower water column), and pelagic (those primarily living upper-middle water column).

Relationship between the DSD and temperature, altitude, and latitude of the distributions of Cyprinidae fishes

We collected these data on Cyprinidae fishes from FAUNA SINICA (Chen 1998; Yue 2000), including SOLL (the number of scales on the lateral line), SALL (the number of scales above the lateral line), SBLL (the number of scales below the lateral line), TS (transverse scales), LS (longitudinal scales) (the mean of the maximum and minimum values of the variation range) and distribution locations (excluding cavefish, there were 509 species, including a small number of subspecies). The latitude, longitude, and altitude data of the river near the distribution location were obtained through Google Earth. Finally, the latitude, longitude, and altitude data were imported into Earth Systems Modelling Results (https://www.paleo.bristol.ac.uk/ummodel/scripts/html_bridge/clamp_UEA.html) to obtain the distribution of air temperature information. If some fish had multiple distribution locations, their mean value was used. The summarized data can be referred to Supplementary File 3. We subsequently performed unary linear regression and Pearson's correlations between the DSD and mean temperature, mean temperature in warm months, and mean temperature in cold months, altitude and latitude of Cyprinidae fishes in China through Origin software (version 2019b, Origin Lab Corporation, Northampton, USA) and IBM SPSS Statistics (version 21.0, Armonk, New York, USA).

Comparison of swimming performance of common carp vs. scaleless carp

To test the effect of scale loss on the swimming ability of common carp *C. carpio*, we selected 10 common carp (standard length, 72.50 ± 4.22 mm, 67–80 mm) and 10 scaleless carp (*C. carpio* scaleless var.) (standard length, 73.40 ± 3.31 mm, 67–79 mm) to test two related indicators of swimming performance. Critical swimming speed (U_{crit}, i.e., the water velocity at which a fish stops swimming in an incremental velocity test) is a widely used parameter for the evaluation of swimming performance in fishes (Reidy et al. 2000; Lee et al. 2003; Farrell 2008). The active metabolic rate reflects the oxygen consumption rate of fish while swimming at a specific water velocity (Pang et al. 2013; Norin and Clark 2016; Pang et al. 2021). During the experiment, we increased the flow rate in the swim tunnel by 6 cm/s every 20 min. The oxygen concentration (mg L⁻¹) in the water was recorded once every 2 min in closed mode by an oximeter (HQ20, Hach Company, Loveland, CO, USA),

and the entire experimental process is detailed in previous studies (see Supplementary Methods 1 for details.) (Pang et al. 2021).

Statistical analyses

IBM SPSS Statistics (version 21.0, Armonk, New York, United States) was used for the data analyses. The mean ± standard deviation (SD) was used to represent the unannotated quantitative data, and the other data were annotated in the table or graph notes. One-way analysis of variance (ANOVA) was followed by multiple comparison tests (Tukey's test) for different taxon groups. Pearson's correlation was used to analyze the relationship between the DSD and other data; $0.0 < |r| \leq 0.25$ indicated no correlation, $0.25 < |r| \leq 0.5$ indicated a weak correlation, $0.5 < |r| \leq 0.75$ indicated a moderate correlation, and $0.75 < |r| \leq 1.0$ indicated a strong correlation (Bae et al. 2006). To determine the impact of phylogeny on the relationships between the DSD and the CSI, we performed phylogenetic independent contrasts (Felsenstein 1985) to determine whether strong relationships existed between them without the influence of phylogeny. Phylogenetic independent contrasts were completed by the APE package (Paradis et al. 2004) in R software. To determine the impact of phylogeny on the ANOVA between the CSI of fishes at different water column positions, we performed phylogenetic ANOVA (Garland et al. 1993). Phylogenetic ANOVA was completed by the phylANOVA function of the phytools package (Revell 2012) in R software. All graphs were generated by Origin software.

Results

Relationship between the DSD and the relative scale thickness

For scales, there was a strong negative correlation between the relative scale thickness and the DSD in all fishes ($r = -0.88$, $P < 0.001$). There were similar results in Cyprinidae fishes ($r = -0.84$, $P < 0.001$) and non-Cyprinidae fishes ($r = -0.88$, $P < 0.001$) (Figure 2).

The phylogenetic relationship of experimental fishes

In Cyprinidae, we selected 33 species belonging to 31 different genera. In the phylogenetic tree, the genetic distances between them were relatively great, which indicated that the genetic relationships of the samples were relatively dispersed. The following species with a higher DSD (DSD > 50) originated independently 5 times: (*Schizothorax wangchiachii* 22, *S. davidi* 23), (*Gymnocypris eckloni* 24), (*Hypophthalmichthys molitrix* 20, *Aristichthys nobilis* 21), (*Elopichthys bambusa* 28) and (*Tinca* 29, *Rhynchocypris lagowskii* 25). Compared with non-Cyprinidae fishes, the CSIs of Cyprinidae fishes were relatively low. For the water column position, most of the species were sub-benthic fishes (N = 24), with fewer pelagic species (N = 5) that originated independently 5 times ((*Hypophthalmichthys molitrix* 20, *Aristichthys nobilis* 21), (*Culter alburnus* 7), (*Hemiculter leucisculus* 10) and (*Elopichthys bambusa* 28, *Opsariichthys bidens* 32)) and benthic fishes (N = 3) that originated independently twice ((*Garra orientalis* 31) and (*Pseudogobio vaillanti* 17, *Abbottina rivularis* 15)) (Figure 3 and Supplementary Tables S1–S2).

For non-Cyprinidae fishes, we selected 21 species with long genetic distances that belonged to 15 different families and 20

different genera. Most species had a higher DSD, and the following species with a lower DSD (DSD ≤ 50) originated independently 6 times: (*Ictiobus Cyprinellus* 40, *Myxocyprinus asiaticus* 41), (*Beaufortia kweichowensis* 36), (*Rhinogobius giurinus* 47, *Odontobutis obscurus* 44), (*Channa argus* 43), (*Micropterus salmoides* 46) and (*Oreochromis niloticus* 42, *Tylosurus melanotus* 54). The DSD and CSI in the non-Cyprinidae fishes were generally covariant. Most species were benthic fishes (*N* = 13), with fewer pelagic species (*N* = 2) that

originated independently twice ((*Ictiobus Cyprinellus* 40) and (*Tylosurus melanotus* 54)) and sub-benthic fishes (*N* = 6) that originated independently 4 times ((*Myxocyprinus asiaticus* 41), (*Esox lucius* 51, *Oncorhynchus mykiss* 52) and (*Siniperca scherzeri* 45, *Micropterus salmoides* 46, *Oreochromis niloticus* 42)) (Figure 3 and Supplementary Tables S1–S2).

Relationship between the DSD and skin structure

For the epidermis and dermis, there was a medium positive correlation between the relative epidermis thickness and the DSD in all the fishes ($r = 0.64$, $P < 0.001$). There was a higher positive correlation in non-Cyprinidae fishes than in all fishes combined ($r = 0.70$, $P < 0.001$). However, there was no correlation in Cyprinidae fishes alone ($r = 0.10$, $P = 0.600$) (Supplementary Figure S1a). Similar relationships existed between the DSD and the relative dermal thickness or relative epidermis and dermal thickness (Supplementary Figure S1b–S1c).

For mucous and club cells, there was a weak positive correlation between the relative total area of mucous cells and the DSD in all fishes ($r = 0.40$, $P = 0.003$ and in non-Cyprinidae fishes ($r = 0.37$, $P = 0.100$), and there was no correlation in Cyprinidae fishes ($r = -0.13$, $P = 0.480$) (Supplementary Figure S1d). However, there was a moderate positive correlation between the relative total area of club cells and the DSD in all fishes ($r = 0.65$, $P < 0.001$ and in non-Cyprinidae fishes ($r = 0.76$, $P < 0.001$). Again, there was no correlation in Cyprinidae fishes ($r = 0.07$, $P = 0.700$) (Supplementary Figure S1e). Similar relationships existed between the relative total area of mucous and club cells and the DSD (Supplementary Figure S1f).
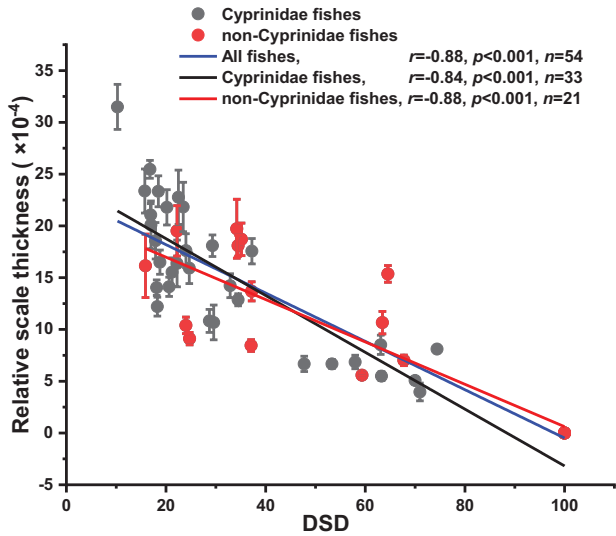

Figure 2 Relationship between the degree of scale degradation (DSD) and relative scale thickness of experimental fishes. The circle and whiskers indicate the mean ± *SE*.
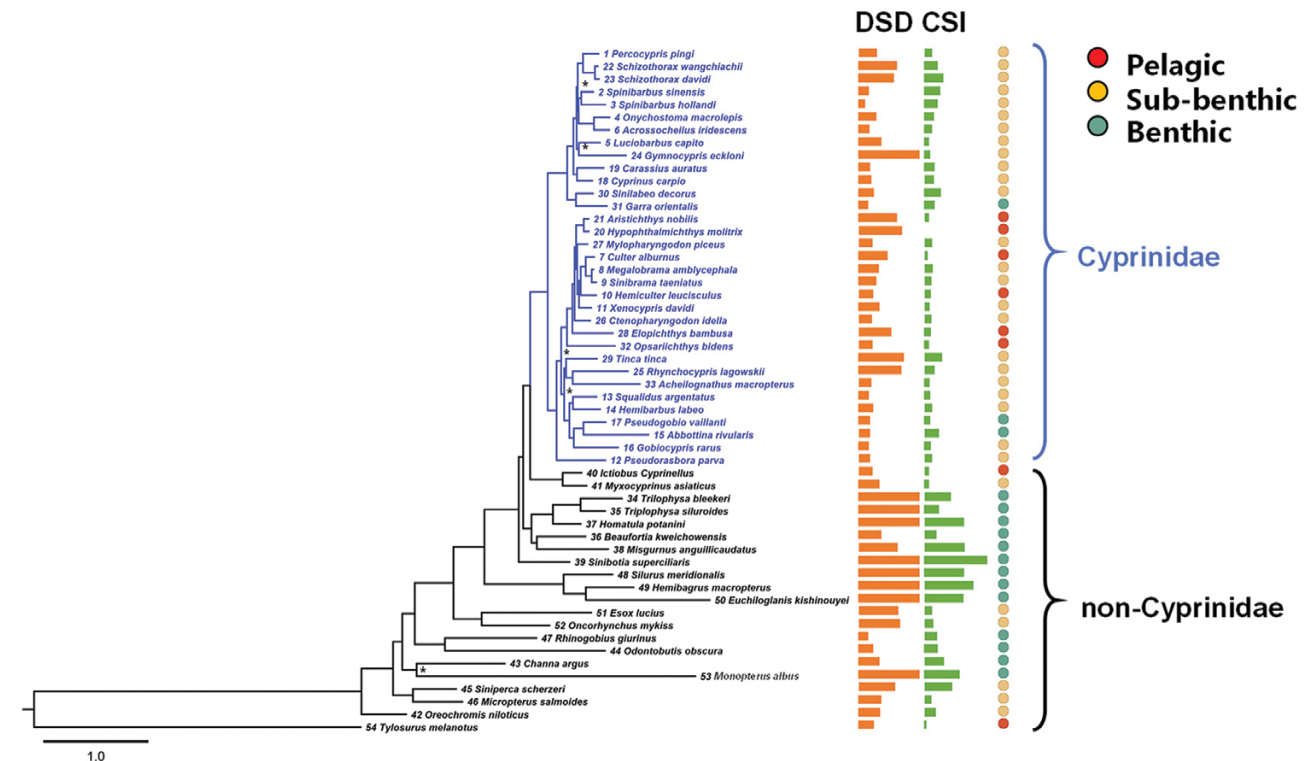

Figure 3 The phylogenetic relationship, degree of scale degradation (DSD), the comprehensive skin index (CSI) and habitat water layer of experimental fishes. The CSIs are standardized to 0–100 based on minimum and maximum values in all species. * represents a bootstrap value of less than 70.

For the comprehensive skin structure, there was a moderate positive correlation between the CSI and the DSD in all fishes ($r = 0.64$, $P < 0.001$). There was a higher positive correlation in non-Cyprinidae fishes than in all fishes combined ($r = 0.73$, $P < 0.001$). However, there was no correlation in Cyprinidae fishes ($r = 0.03$, $P = 0.860$) (Figure 4). We established separate phylogenetic trees for all fish, Cyprinidae fishes and non-Cyprinidae fishes for phylogenetic independent contrasts analysis. After phylogenetic correction, the correlation between the CSI contrasts and the DSD contrasts in all fishes decreased, showing a weak positive correlation ($r = 0.32$, $P = 0.020$). There was still no correlation in Cyprinidae fishes ($r = -010$, $P = 0.585$). However, the correlation between the CSI contrasts and the DSD contrasts in non-Cyprinidae fishes increased, showing a strong positive correlation ($r = 0.80$, $P < 0.001$).

Relationship among the habitat layers, DSD and CSI

All fish were divided into pelagic, sub-benthic, and benthic groups according to their habitat requirements (Figures 5A and 3 and Supplementary Table S1). In pelagic fishes, there was a weak negative correlation between the DSD and the CSI ($r = -0.40$), but the number of samples was small ($N = 8$), which may be the reason why there was no significant difference ($P = 0.330$). In sub-benthic fishes, there was no correlation between the DSD and the CSI ($r = 0.24$, $P = 0.200$). In benthic fishes, there was a strong positive correlation between the DSD and the CSI ($r = 0.77$, $P < 0.001$) (Figure 5A). In addition, the CSIs of each section of fish in different habitat layers were compared. The CSI of benthic fishes (1.73 ± 0.21) was significantly higher ($P < 0.01$) than that of sub-benthic fishes (-0.58 ± 0.05), and the latter was significantly higher ($P < 0.01$) than that of pelagic fishes (-1.32 ± 0.05). In phylogenetic ANOVA, the CSI of benthic fishes was significantly
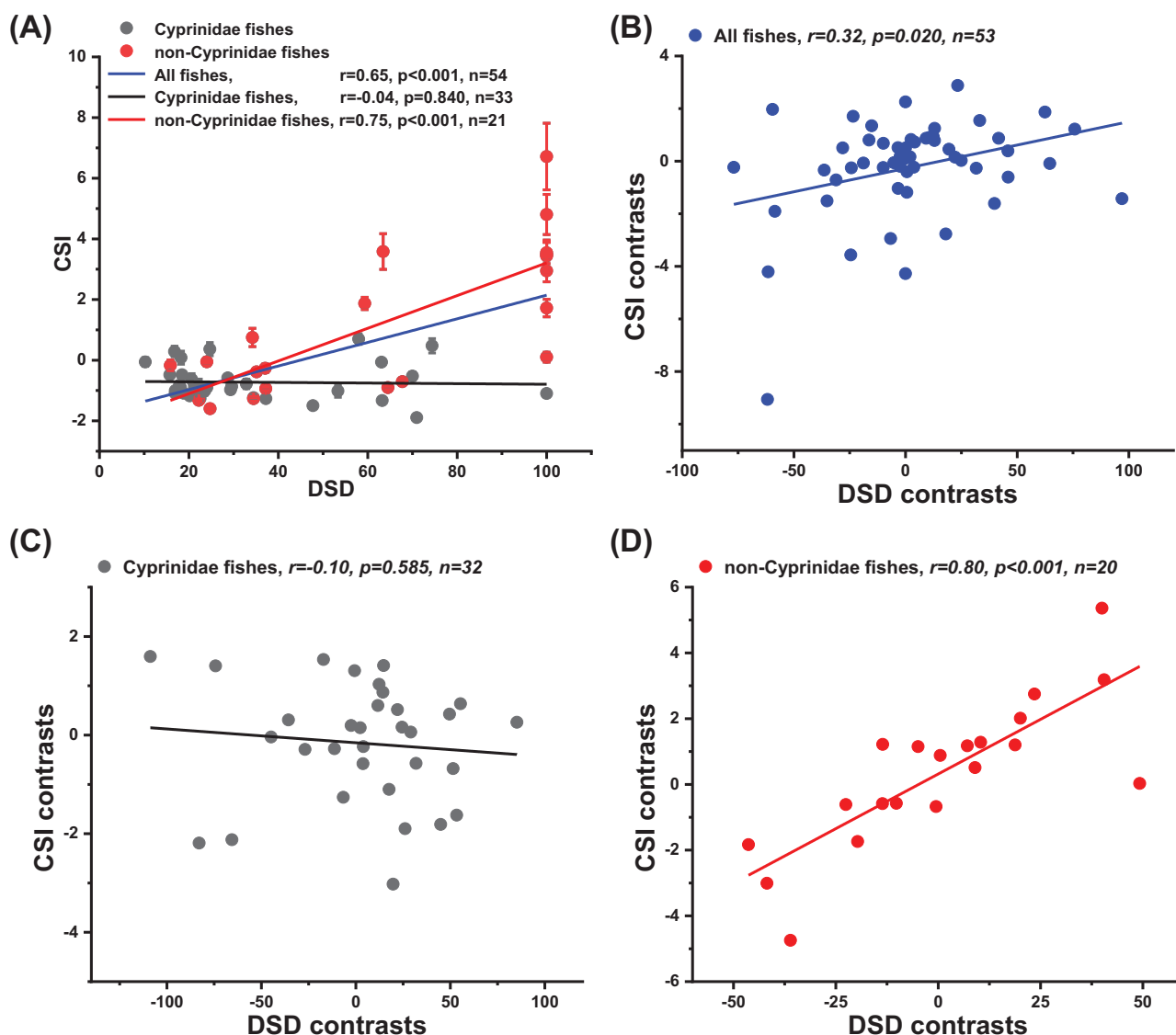
Figure 4 Relationship between the degree of scale degradation (DSD) and the comprehensive skin index (CSI) of experimental fishes. (A) Traditional correlation between the DSD and CSI of experimental fishes. (B) Corrected correlation between the DSD and CSI of all fishes by phylogenetic independent contrasts (PIC). (C) Corrected correlation between the DSD and CSI of Cyprinidae fishes by PIC. (D) Corrected correlation between the DSD and CSI of non-Cyprinidae fishes by PIC. The circle and whiskers indicate the mean ± *SE*.

higher ($P < 0.01$) than that of sub-benthic fishes and pelagic fishes, and there was no significant difference between them ($P = 0.206$) (Figure 5B).

We also found that some pelagic fishes with more scales, such as *Hypophthalmichthys molitrix* (20) and *Elopichthys bambusa* (28) (Figure 5C) were also larger in adult size. To confirm this finding, we collected the DSD data of 46 species of pelagic fishes from FAUNA SINICA. Species with high-altitude distributions (average altitude > 1,000 m) were excluded, as the results in the next section indicate that altitude has a significant influence on the number of fish scales. We referred to previous studies (Ding 1994; Chen 1998; Yue 2000; Guo et al. 2021) and our laboratory's long-term field investigation experience in the classifications of the water column position and adult size. The results showed that the DSD of the larger pelagic fishes ($N = 23$, 43.68 ± 2.61, 30.62–70.79) was significantly higher ($P < 0.001$) than that of the small pelagic fishes ($N = 23$, 23.31 ± 0.46, 19.53–27.93).

Relationship between the DSD and temperature, altitude, and latitude of the distributions of Cyprinidae fishes

The results showed that the DSD of Cyprinidae fishes had a stronger negative correlation with the mean temperature in warm months ($r = -0.684$, $P < 0.001$) than with the mean temperature ($r = --0.518$, $P < 0.001$) and the mean temperature in cold months ($r = -0.361$, $P < 0.001$) (Figures 6A, B). The DSD of Cyprinidae fishes exhibited a higher correlation with altitude ($r = 0.711$, $P < 0.001$) than with latitude ($r = 0.186$, $P < 0.001$) (Figures 6C, D).

Comparison of swimming performance of common carp vs. scaleless carp

There was no significant difference in the U_{crit} and U_{crit}/BL (body length) between common carp and scaleless carp (Figures 7A,B). There was no significant difference in active metabolic rate between common carp and scaleless carp at any swimming speed (Figure 7C).
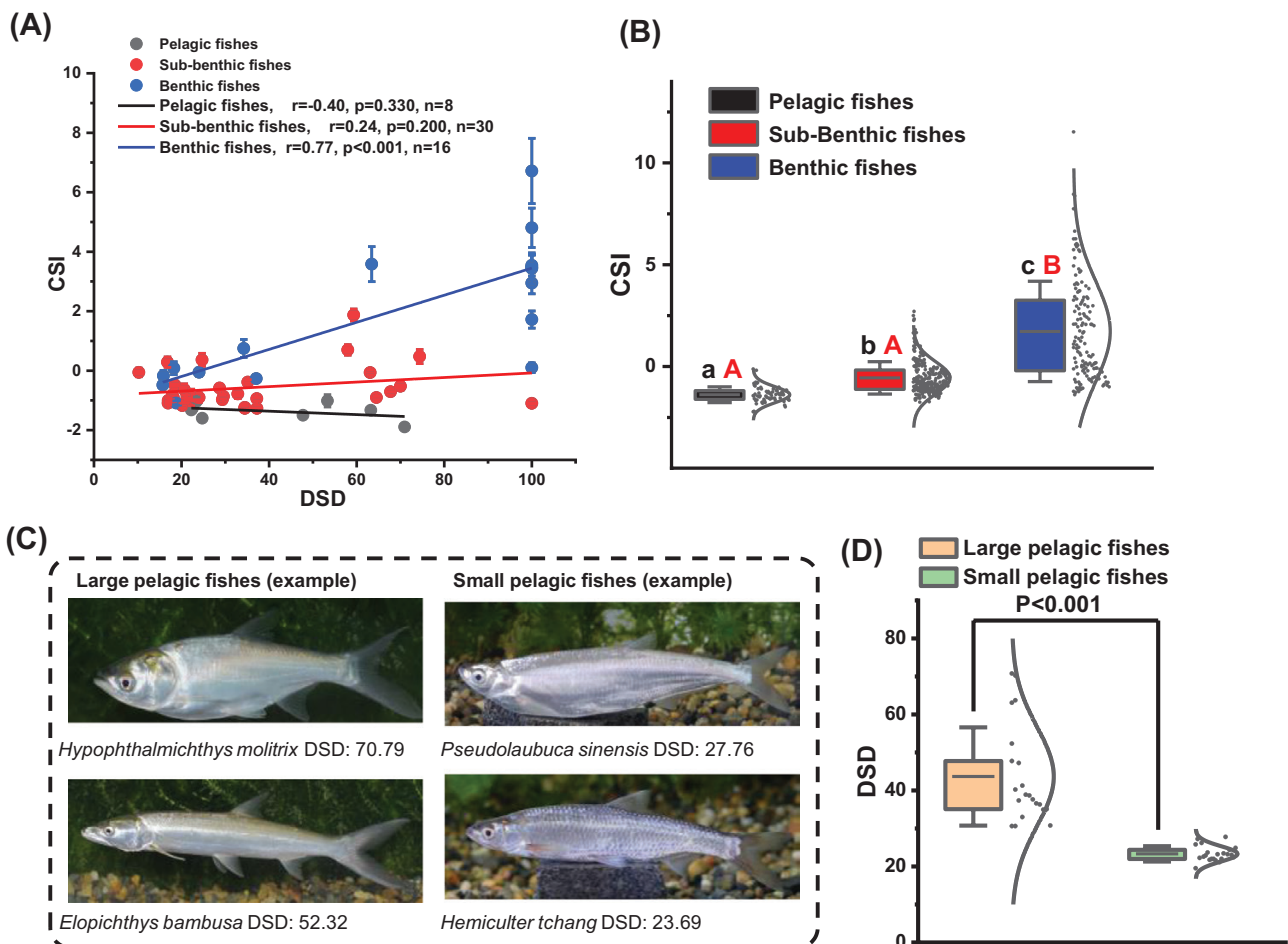

Figure 5 Relationship among the habitat layers, the degree of scale degradation (DSD) and the comprehensive skin index (CSI) of experimental fishes. (A) Relationship between the DSD and CSI of experimental fish in different water column positions. (B) Comparison of the CSI of fish in different water column positions. (C) Examples of large and small pelagic fishes. (D) Comparison of the DSD of pelagic fishes with different body types. In (B), each dot represents data for each tissue image of each species. The different superscripts differ (A, B) significantly at $P < 0.01$ based on Tukey test, the different superscripts differ (A, B) significantly at $P < 0.05$ based on Phylogenetic ANOVA, and whiskers indicate mean ± SE. Figure (C) from Guo et al. (2021).
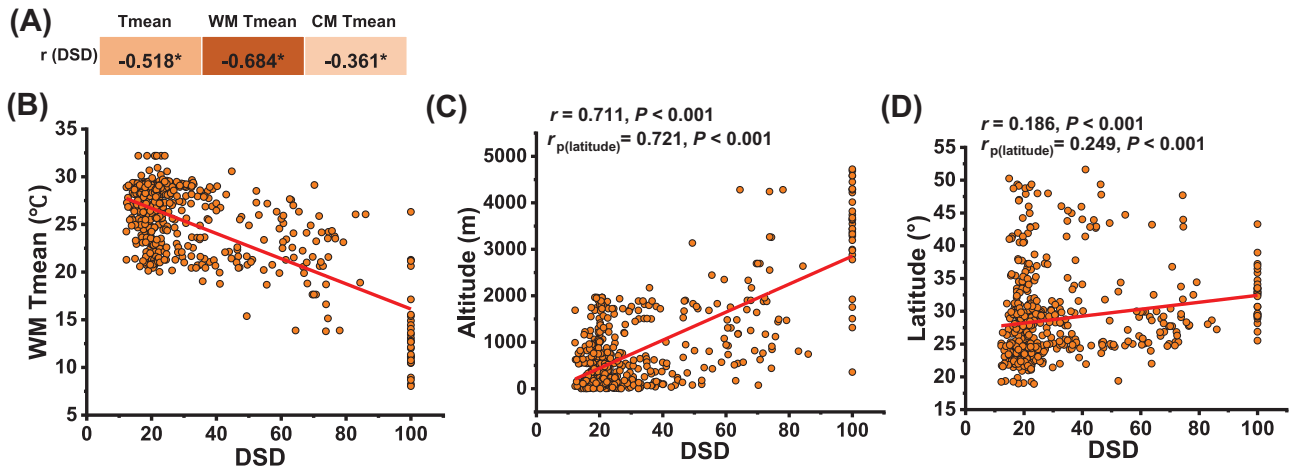
Figure 6 Relationship between the degree of scale degradation (DSD) and temperature, altitude, and latitude in Cyprinidae fishes. (A) Correlation analysis between the DSD and mean temperature (T_{mean}), mean temperature in warm months (WM T_{mean}), and mean temperature in cold months (CM T_{mean}). * represents $P < 0.001$; r represents Pearson's bivariate correlation analysis. (B) Relationship between the DSD and WM T_{mean}. (C) Relationship between the DSD and altitude. (D) Relationship between the DSD and latitude. r and r_p indicate Pearson's correlation analysis and partial correlation analysis with latitude or altitude as a control variable, respectively.
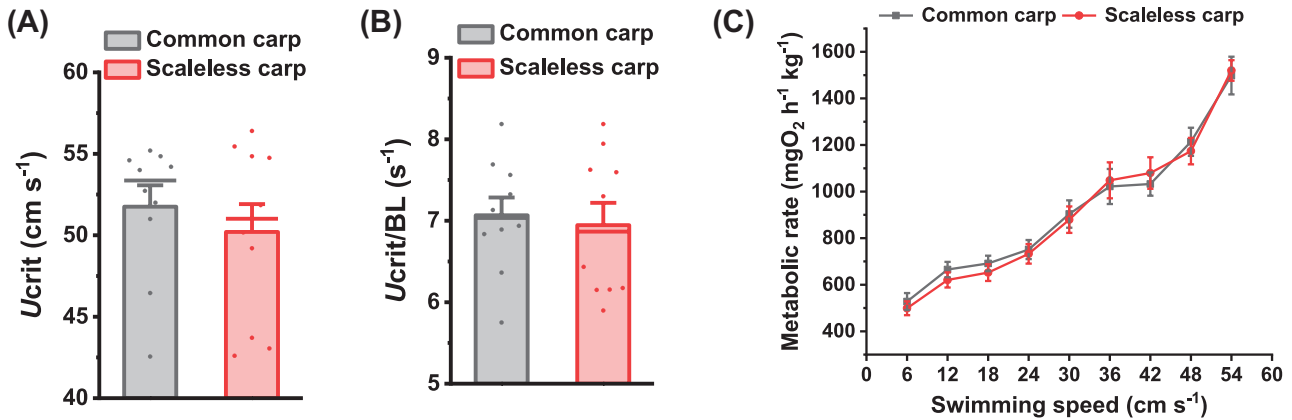

Figure 7 Comparison of swimming performance between common carp and scaleless carp. (A) Comparison of critical swimming speed (U_{crit}) between common carp and scaleless carp. (B) Comparison of critical swimming speed relative to body length (U_{crit}/BL) between common carp and scaleless carp. (C) Relationship between swimming speed and metabolic rate of common carp and scaleless carp. The height gives the mean, the thick lines give the medians, and whiskers indicate mean ± *SE*.

Discussion

Regardless of the composition of scales, the scale thickness can directly reflect its protective function. However, there is a lack of interspecific study on the relationship between the scales number and scales thickness in fish, so functionally, it does not directly indicate that the smaller or more numerous scales are degenerate. In this study, we found that there was a strong negative correlation between the scale thickness and scale number, indicating that the more fish scales there were, the more degraded the scales were. In addition, we also proved that quantification of scale degradation by DSD is feasible. To explore the antagonistic relationship between scales and skin development on protective function, this study proposed the CSI as an indicator to quantify the protective function of the skin. In traditional correlation analysis, the moderate positive correlation between the CSI and the DSD in 54 fish species supported this hypothesis, especially in non-Cyprinidae fishes (Figure 4). Interestingly, in the phylogenetic independent contrasts correlation analysis, the correlation coefficient (r) between the CSI and the DSD in non-Cyprinidae fishes was slightly increased, but this correlation coefficient (r) in all fishes showed a greater decline. This

phenomenon may be caused by the asymmetric distribution of phylogenetic taxa (Felsenstein 1985); that is, there are many species of Cyprinidae fishes, but non-Cyprinidae fishes have fewer and more dispersed species in the phylogenetic tree. However, there was no correlation between the DSD and the CSI in Cyprinidae fishes, either traditional or phylogenetic independent contrasts correlation analysis.

The CSI of the fishes was related to their water column position with the value of benthic fishes being highest and that for epipelagic fishes lowest (Figures 5A,B). This is easy to explain because the function of the skin is mainly to protect and secrete mucus to reduce friction with the benthic substrate and avoid friction from causing damage to the skin (Elliott 2000; Xie 2009). The epipelagic fishes generally do not rub against rocks or sediment, especially filter-feeding fish such as silver carp and bighead carp, so they do not need thick skin or more mucus or other secretions to resist friction. The sub-benthic fishes tend to prey on aquatic plants and benthic animals or ambush smaller fish, so they may have more friction with surrounding objects and may need a moderately well-developed protection function of the skin. The benthic fishes tend to live on the bottom of the riverbed or

in the sediment and have much friction with the surrounding substrate, such as catfish and loach, so they need a well-developed protective function of the skin to reduce friction.

However, we found that the DSD showed a significant positive correlation with the CSI only in the benthic fishes (Figure 5A). This finding showed that scale degradation may be related to the enhanced protective function of skin only in benthic fishes. Indeed, scale degradation can make their bodies more flexible and adaptive for cave and benthic life. However, there was a lack of typical benthic layer fishes with well-developed skin function in Cyprinidae. *Abbottina rivularis* (15), *Pseudogobio vaillanti* (17) and *Garra orientalis* (31) were defined as benthic fishes in this study, but they tend to live in riverbeds and do not have the same burrowing behavior as loaches. In summary, we considered the lack of typical benthic fishes in Cyprinidae to be an important reason for the poor correlation between the DSD and the CSI. Second, our research also showed that in addition to benthic adaptations, there are other factors that affect the number of fish scales.

In the above results, we found that benthic adaptation was an important cause of scale degradation in fishes. However, the cause of scale degradation in Cyprinidae fishes has not been found. The fishes that inhabit the Tibetan Plateau are mainly fine-scale or scaleless species, which provides important clues. Furthermore, we collected the ecological habitat factors and the DSD of 509 species of Cyprinidae fishes in China. We found a significant negative correlation between the DSD and the mean temperature, especially in warm months (Figure 6a). Both latitude and altitude can affect temperature, but latitude had a greater impact on the DSD in our results (Figures 6b–c). Altitude mainly affects the annual mean temperature, while latitude mainly affects the mean temperature in cold months. Therefore, the reason for the above results may be because fish mainly grow in warmer months. In summary, the decrease in temperature caused by the increase in altitude was an important factor in the scale degradation of Cyprinidae fishes in China.

Pang et al. (2021) proposed that U_{crit} and active metabolic rate were two indicators that reflect swimming performance. The scaleless carp has a smoother and more supple integument than does the common carp, which may lead to a reduction in its swimming resistance. Therefore, we hypothesized that scaleless carp had a better swimming performance (a higher U_{crit} and a lower active metabolic rate as the resistance of the fish's body decreases, resulting in less energy consumption) than the common carp. However, we did not find a difference in the U_{crit} or the metabolic rate at different swimming speeds between them (Figure 4), indicating that their swimming performance was similar. It may have also been that the benefit of scale loss to swimming performance was small and the significance was offset by individual differences. Therefore, steady swimming costs do not appear to be affected by fish scales, which is similar to the results of swimming comparisons of pike (*Esox sp.*, with small scales) and gar (*Lepisosteus osseus,* with large scales) (Webb et al. 1992).

Some pelagic fishes have both degenerate scales and skin. Both scales and skin provide mechanical protection, but the lack of obstacles such as rocks in the upper water makes them less important. Interestingly, we found that the scales of the larger pelagic fish were more degenerate than those of the smaller pelagic fish (Figure 5e). Small pelagic fish mostly live in small waterbodies, and there are more interactions with the substrate than in large waterbodies. In contrast, large pelagic fishes live more in large rivers or lakes, which have fewer interactions with the substrate. Therefore, we speculate that

in the pelagic water column with less substrate interaction, large pelagic fishes reduce their investment in scales and skin, resulting in scale and skin degradation.

In summary, our results show that there is a rich diversity of skin structure in fishes, and fish with more well-developed skin tend to have degenerate scales, but fish with degenerate scales do not necessarily have well-developed skin. Diverse factors lead to the degeneration of fish scales. This study summarized three factors that affect the number of scales: low temperature, benthic adaptation, and pelagic adaptation. Only in benthic fishes, the more well-developed their skin, the more scales. These interspecific differences related to the lifestyle of these fishes are based on their associated habitats and are an outcome of long-term selection.

Author Contributions

H.R.G. and Z.J.W. conceived the ideas and designed this study. H.R.G. wrote the original manuscript and completed the data analysis. H.R.G., H.Y.W., and S.D.Z. completed histology experiments. H.R.G., Z.J.W., D.Y.Y., and X.Y.D. participated in the discussion of the experimental results and the improvement of the manuscript.

Ethics Statement

All the animal procedures were conducted with the approval of the Committee of Laboratory Animal Experimentation at Southwest University, Chongqing, China and were in full compliance with the Committee's guidelines [protocol number (2014)25].

Conflict of Interest

The authors declare that they have no competing interests.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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