Holding in the stream – convergent evolution of suckermouth structures in Loricariidae (Siluriformes)

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Abstract

Suckermouth armoured catfish (Loricariidae) are a highly specious and diverse freshwater fish family, which bear upper and lower lips forming an oral disc. Its hierarchical organisation allows the attachment to various natural surfaces. The discs can possess papillae of different shapes, which are supplemented, in many taxa, by small horny projections, i.e. unculi. Although these attachment structures and their working mechanisms, which include adhesion and interlocking, are rather well investigated in some selected species, the loricariid oral disc is unfortunately understudied in majority of species, especially with regard to comparative aspects of the diverse oral structures and their relationship to the ecology of different species. In the present paper, we investigated the papilla and unculi morphologies in 69 loricariid species and undescribed taxa, which inhabit different currents and substrates. We determined four papilla types and eight unculi types differing by forms and sizes. From handling the structures and from drying artefacts we could infer some information about their material properties. This, together with their shape, enabled us to carefully propose hypotheses about mechanisms of interactions of oral disc structures with natural substrates typical for respective fish species.

Background

The suckermouth armored catfishes (Loricariidae, Siluriformes, Teleostei) are among the most interesting animal groups, because this family is, with about 1,000 species [1], highly specious as a result of an evolutionary radiation, which included convergent evolution of traits [2, 3]. These neotropical freshwater fish are particularly diverse with regard to body colorations and shapes, reflecting their high degree of specialisation to different habitats and speciation.

Loricariidae are characterized by a depressed body shape covered by bony plates and the modification of the mouth to an oral sucker disc, which is used, besides for feeding, for the attachment to various substrates [2, 4, 5, 6, 7]. In specialized species, it also facilitates climbing [8]. This oral disc, which can only be found in three more catfish families (Astroblepidae, some genera in Mochokidae and in Sisoridae), is an adaptation to fast flowing water bodies and is used to generate negative pressure to allow surface attachment [6]. The disc is spiked with small papillae, which are probably used for foraging and increasing the attachment capability by friction to reinforce the seal of the disc [9, 10, 11, 12, 13]. In some taxa, horny projections of single cells, i.e. unculi, can be found on top of the papillae, which are highly diverse in morphology among fish [14]. In Loricariidae, they are potentially involved in feeding [10, 12], but probably increase the friction during attachment as well [6, 11, 13, 14, 15, 16, 17].

In fish taxa, such as Balitoridae, Gobiesocidae, Cyclopteridae, Liparidae, Echeneidae, and Cyprinidae, attachment structures and attachment mechanisms have been previously investigated [18,19,20,21,22,23,24,25,26,27,28; see reviews by 29,30,31,32].

But for Loricariidae, besides of the above-mentioned references, little is known about the structures of the suckermouth and of the oral papillae, even though they are highly diverse [2, 3, 7, 14, 33]. We here study
the morphology of papillae and unculi of 69 species and undescribed taxa from all kinds of habitats to pave the way for more research addressing the diversity of oral discs. According to their shape, the structures were sorted to different categories. Although, comparative studies on the attachment performance of these fish taxa are lacking, we present hypotheses on the interaction between mouth and substrate based on the literature on attachment structures in different animal taxa.

Results

Morphology of papillae

On the lower lips (Fig. 1A–D), we could differentiate four papilla types (Fig. 1E–H):

1. Flat papillae

This papilla was broad with a flattened tip (Fig. 1E). Each papilla measured between 100–200 µm in diameter and had a height of approximately 50 µm. Up to 20 papillae were found on each studied area, depending on the size of the lip. When this papillae type was manipulated, the bases seemed relatively flexible and the tip rather stiff. This pattern was detected in Ancistrus ranunculus, Pseudacanthicus pitanga, Crossoloricaria cephalaspis, Loricaria luciae, L. simillima, Pseudohehiodon almendarizi (Fig. 1E), Pterosturisoma microps, and Spatuloricaria puganensis.

2. Short papillae

Papillae were rather roundish in profile (Fig. 1F). They were of 100–200 µm diameter and 70–100 µm in height. When papillae were manipulated, the tip seemed rather flexible whereas the basis seemed rather stiff. This kind was determined for Hypoptopoma inexpectatum, Ancistrus sp. L464 (Fig. 1F), Chaetostoma formosae, Scobinancistrus aureatus, and Hemiloricaria melini.

3. Medium papillae

Papillae were roundish and of medium height (300–400 µm) and of 100–300 µm diameter (Fig. 1G). The tip seemed rather flexible, but the basis rather stiff. We observed these papillae in Otocinclus cocama, Rhinotocinclus isabelae, Acanthicus adonis (Fig. 1G), Ancistomus cf. sabaji, A. spilomma, Ancistrus cirrhosus, A. dolichopecterus, A. luzia, A. sp. L107, A. sp. L519, Baryancistrus aff. niveatus, B. xanthellus L177, B. xanthellus L81, Chaetostoma dorsale, C. lineopunctatum, Dekeyseria picta, Guyanancistrus brevispinis, Hypancistrus contradens, H. inspector, H. sp. L174, H. zebra, Hypostomus cochliodon, Leporacanthurus joselmae L264, L. sp. L240, Oligancistrus immaculatus, Panaqolus sp. L271, P. sp. L351, Panaque nigrolineatus L190, Parancistrus nudiventris L31, P. nudiventris L258, P. nudiventris L300, Pseudacanthicus sp. L97, P. sp. L185, P. sp. L273, P. sp. L65, P. spinosus, Pseudolithoxus dumus, Scobinancistrus aff. pariolispos, Cteniloricaria platystoma, Farlowella oxyrryncha, F. platynychus, Hemiodontichthys acipenserinus, Lamontichthys filamentosus, L. stibaros, Sturisomatichthys aureus, and S. festivus.
4. Long papillae

Papillae were roundish and long (500–550 µm) and of 80–100 µm diameter (Fig. 1H). The tip seemed rather flexible, but the basis rather stiff. We detected long papillae in Ancistrus claro, A. megalostomus, Aphanotorulus cf. emarginatus, Hypancistrus sp. L333 (Fig. 1H), Hypostomus bolivianus, H. laplatae, Parancistrus aurantiacus, Peckoltia sp. L76, Scobinancistrus sp. L82, Pseudorinelepis cf. genibarbis L152, P. cf. genibarbis L95, and Rhinelepis aspera.

**Morphology of unculi**

We could not detect a high degree of intraspecific variability in species with more than one examined specimen (Supplementary Fig. 2). Overall, we differentiated eight types of morphology related to the unculi on the papillae surfaces (see below) (Fig. 2).

1. No unculi

Here, we could not detect small projections but a rather bulky surface of the papillae (Fig. 2A). The surface seemed rather stiff. We observed this in Rhinotocinclus isabelae, Ancistrus ranunculus, Dekeyseria picta, Hypancistrus sp. L174, H. zebra, Leporacanthicus joselimai, L. sp. L240, Oligancistrus immaculatus, Parancistrus aurantiacus, P. nudiventris L31, P. nudiventris L258, P. nudiventris L300, Crossoloricaria cephalaspis, Loricaria luciae, L. simillima, and Pseudohemiodon almendarizi (Fig. 2A).

2. Suction cups (no unculi)

On the central surface of the papilla, a round to oval structure with a thick outer bulge and an inner depression could be determined (Fig. 2B). The papillae themselves varied from 150 µm to 200 µm in diameter and the structure were of about 50 µm in diameter. The tip of the papilla seemed flexible and the basis rather stiff. This surface structure was detected in Acanthicus adonis (Fig. 2B).

3. Elongated unculi with hook-like tips

Long projections of 15–20 µm length situated in the center of each papilla (Fig. 2C). Each papilla hosted 20–40 single unculi, depending on the papillae size. The tips of the unculi are formed like a hook and most of them are pointed into the same direction. Tips of the hooks seemed rather stiff, whereas the bases seemed rather flexible. We found these unculi in Ancistrus sp. L464 (Fig. 2C) and Lamontichthys stibaros.

4. Mushroom-like unculi

Here, elongated unculi of 15–25 µm with flattened tips were found (Fig. 2D). Papillae were entirely covered with 25–40 unculi, depending on the size of the papillae. The tips seemed rather stiff, whereas the bases seemed rather flexible. This was observed in Otocinclus cocama, Ancistrus cirrhosus, A. claro, A. luzia, A. sp. L107, A. sp. L519, Chaetostoma dorsale, C. formosae (Fig. 2D), C. lineopunctatum, Scobinancistrus aureatus, S. sp. L82, Cteniloricaria platystoma, Farlowella oxyryynchus, F. platorynchus,
Lamontichthys filamentosus, Sturisomaticthys aureus, Pseudorinelepis cf. genibarbis L152, and P. cf. genibarbis L95.

5. Small mushroom-like unculi

In some species, unculi of 10–15 µm length with flattened tips were found (Fig. 2E). Papillae were covered with 10–15 unculi. The tips seemed rather stiff, whereas the bases seemed rather flexible. We found this type of unculi in Hypancistrus contradens, Hemiloricaria melini, Hemiodontichthys acipenserinus, and Spatuloricaria puganensis (Fig. 2E).

6. Honey-combed pattern

Here, the whole papilla surface (150–200 µm diameter) pattern was reticulated (Fig. 2F). The parts of the structure interacting with the target surface seemed rather flexible and the bases rather stiff. This type was determined for Ancistrus megalostomus (Fig. 2F).

7. Long filamentous unculi

Unculi were thin (~ 1 µm thick) and of 10 µm length (Fig. 2G). On each papilla, 30–40 single filaments were found. They seemed very flexible. This surface pattern was detected in Hypancistrus sp. L333, Pseudacanthicus pitanga (Fig. 2G), P. sp. L97, P. sp. L185, P. sp. L273, P. sp. L65, P. spinosus, Sturisomaticthys festivus, and Rhinelepis aspera.

8. Folds

Unculi were of 800–1000 µm length and of 90–100 µm width (Fig. 2H). On each side of the papilla, unculi were arranged inversely. At the very tip of papilla, the unculi formed a fold. The unculi seemed rather flexible on their bases and stiff at their tips. This pattern was detected in Aphanotorulus cf. emarginatus, Baryancistrus aff. niveatus, B. xanthellus L177, B. xanthellus L81, Hypostomus bolivianus (Fig. 2H), H. laplatae, Panagolus sp. L271, P. sp. L351, Panaque nigrolineatus, Peckoltia sp. L76, and Scobinancistrus aff. pariolispos.

**Relationship between ecology, morphology, and systematic position**

Most of the studied taxa either inhabited rivers of strong or medium current (in both environments, 33 of 69 species were collected) (see Supplementary Table 2). In strong current, most species could be found on stone (27 of 33 species), followed by wood (4 of 33 species), and finally sand (2 of 33 species). Most of the species inhabiting streams with medium current inhabited wood (18 of 33 species), followed by stone (7 of 33 species), sand (6 of 33 species), and both wood and stone (2 of 33 species). Only two species were found in slow flowing water; here the studied species adhered to wood.

In strong currents, most species possessed medium papillae (20 of 33 species), followed by long (6 of 33 species), flat (5 of 33 species), and short papillae (2 of 33 species). Most taxa beard either mushrooms or
no unculi (for each type, 8 of 33 species), followed by long filaments (6 of 33 species), folds (4 of 33 species), honey-combed and hooks (for each type, 1 of 33 species). In medium currents, most species (23 of 33 species) possessed medium-sized papillae, followed by long (4 of 33 species), short and flat papillae (for each type, 3 of 33 species). Here, most species beard mushrooms or folds (for each type, 7 of 33 species), followed by no unculi (6 of 33 species), small mushrooms (4 of 33 species), long filaments (3 of 33 species), hooks and suction cups (for each type, 1 of 33 species). The two species inhabiting slow streams possessed long papillae with mushroom-shaped unculi; these species can be found on wood.

When the substrate is on focus, we find that most species could be found on stone (34 of 69 species), followed by wood (24 of 69 species), sand (8 of 69 species), and finally both wood and stone (2 of 69 species) (see Supplementary Table 3). Most species adhering to stone were found in rivers with strong current (27 of 33 species), followed by medium current (7 of 33 species). Wood as preferred substrate seemed to be mostly inhabited in medium currents (18 of 24 species), followed by strong (4 of 28 species) and slow current (2 of 28 species). Most species inhabiting sand were found in streams with medium (6 of 8 species), followed by strong current (2 of 8 species). Both wood and stone were inhabited in medium currents (2 of 2 species).

Most stone-inhabitants possessed medium papillae (24 of 34 species), followed by long (6 of 34 species), short and flat papillae (for each type, 2 of 34 species). Here, most taxa beard no unculi (8 of 34 species), followed by long filaments and mushrooms (for each type, 7 of 34 species), folds (5 of 34 species), and finally honey-combed and small mushrooms (for each type, 1 of 34 species). When wood was preferred, most taxa possessed medium papillae (15 of 24 species), followed by long (6 of 24 species), short (2 of 24 species), and finally flat ones (1 of 24 species). Most species beard mushrooms (9 of 24 species), followed by folds (6 of 24 species), hooks (2 of 24 species), and finally long filaments, no unculi or suction cups (for each type, 1 of 24 species). Sand-inhabitants mostly possessed flat papillae (5 of 8 species), followed by medium (2 of 8 species) and short ones (1 of 8 species). Here, taxa beard mostly no unculi (5 of 8 species) or small mushrooms (3 of 8 species). The two species adhering to both wood and stones possessed medium papillae with either long filaments or mushrooms (for each type, 1 of 2 species).

With regard to the phylogenetic position (Fig. 3), we found that species inhabiting streams with strong current belonged to Hypostominae, Loricariinae, and Rhinelepiinae. Rivers with medium current were colonized by Hypoptopomatinae, Hypostominae, and Loricariinae. Species living in slow flowing waters belonged to the Rhinelepiinae. In most cases, when more than one species of a genus was studied (Leporacanthicus, Panaqolus, Scobinancistrus, Parancistrus, Ancistomus, Baryancistrus, Chaetostoma, Hypostomus, Loricaria, Lamontichthys, Sturisomaticthys, Farlowella, Pseudorinelepis), we found that the taxa of the same genus preferred the same current type. In some cases (Pseudacanthicus, Hypancistrus, Ancistrus), species of the same genus inhabited different stream types (Fig. 3).
Preference of stone could be determined for Hypostominae, Loricariinae, and Rhinelepiinae (Fig. 3). Wood-preference was found in Hypoptopomatinae, Hypostominae, Loricariinae, and Rhinelepiinae. Sand-dwelling was observed in Dekeyseria (Hypostominae) and some Loricariinae taxa. Both wood and stones were only inhabited by Sturisomatichthys, belonging to Loricariinae. In most cases, when more than one species of a genus was studied (Pseudacanthicus, Leporacanthicus, Panaqolus, Scobinancistrus, Parancistrus, Hypancistrus, Ancistomus, Baryancistrus, Chaetostoma, Hypostomus, Loricaria, Lamontichthys, Sturisomatichthys, Farlowella, Pseudorinelepis), we found that the taxa of the same genus preferred the same substrate. Only in Ancistrus, species adhere to different substrates (Fig. 3).

Flat papillae were detected in Hypostominae and Loricariinae (Fig. 3). The short type was determined for Hypoptopomatinae, Hypostominae, and Loricariinae. Medium sized were identified for Hypoptopomatinae, Hypostominae, and Loricariinae. Large ones were found in Hypostominae and Rhinelepiinae. In most cases, when more than one species of a genus was studied (Leporacanthicus, Panaqolus, Parancistrus, Ancistomus, Baryancistrus, Loricaria, Lamontichthys, Sturisomatichthys, Farlowella, Pseudorinelepis), we found that the taxa of the same genus possessed the same papilla type. In some cases (Pseudacanthicus, Scobinancistrus, Hypancistrus, Ancistrus, Chaetostoma, Hypostomus), species of the same genus showed different types (Fig. 3).

Unculi of the fold type were determined in Hypostominae (Fig. 3). The suction cups were only found in Acanthicus adonis (Hypostominae) and the honey-combed type in Ancistrus megalostomus (Hypostominae). Hooks were detected in Ancistrus sp. L464 (Hypostominae) and Lamontichthys stibaros (Loricariinae). Mushrooms were determined in Hypoptopomatinae, Hypostominae, Loricariinae, and Rhinelepiinae. Small mushrooms were found in Hypancistrus contradens (Hypostominae) and some Loricariinae. Long filaments were detected in taxa of Hypostominae, Sturisomatichthys festivus (Loricariinae), and Rhinelepis aspera (Rhinelepiinae). No unculi were found in Hypoptopomatinae, Hypostominae, and Loricariinae. In most cases, when more than one species of a genus was studied (Pseudacanthicus, Leporacanthicus, Panaqolus, Parancistrus, Baryancistrus, Chaetostoma, Hypostomus, Loricaria, Farlowella, Pseudorinelepis), we found that the taxa of the same genus possessed the same unculi type. In some cases (Scobinancistrus, Hypancistrus, Ancistrus, Lamontichthys, Sturisomatichthys), species of the same genus showed different types (Fig. 3).

Discussion

Attachment structures

Attachment mechanisms are omnipresent in animals, including insects [38, 39, 40, 41, 42, 43, 44, 45, 46], molluscs [47, 48] reptiles [49, 50], amphibians [51, 52], mammals [53, 54] and fishes [26, 55, 56].

These mechanisms are diverse and can include interlocking structures, such as hooks, locks, clamps or spacers [40], wet and dry adhesion [57, 58], and/or suction cups [24, 26]. Depending on the attachment system, physical effects as friction, mechanical interlocking, muscular force, viscous forces, chemical
bonding, capillary effects, van der Waals forces, and electrostatic forces are involved and can lead to permanent, transitory and temporary attachment time to the substrate [40, 59, 60].

With regard to the aquatic environment, two main attachment strategies, bioadhesive secretion or suction attachment, seem to be present as adaptation to the specific physical conditions [see reviews 60,61]. Glue-like bioadhesive secretions include complex mixtures of proteins, lipids and sugars and can be found in echnioderms, mussels, or barnacles. Suction attachment involves muscular contraction to generate pressure differences and can be found in cephalopods, some insect taxa, and fish. In some species, both mechanisms can be found, as in lottiid limpets or fish. Depending on the taxa, attachment is achieved by multiple points of interaction, as in Echniodermata or Cephalopoda, or by one single attachment point, as in limpets or fish [see review 61].

In fish, the attachment structure, i.e., the suction disc, represents a chamber of subatmospheric pressure, to create adhesion by suction to various substrates [7, 11, 18, 23, 25, 26, 55, 56, 62], which can even enable climbing vertical surfaces outside the water column [8, 20, 63, 64, 65].

Performance of the fish sucker depends on many different factors of the attachment structure itself, such as muscle contraction, kinematics, material properties, size, and shape [18, 24, 25, 31, 56, 66, 67, 68, 69, 70, 71]. Additionally, the fish attachment ability is affected by the intensity of the water stream [72, 73] and the substrate curvature and its surface properties [11, 22, 25, 26, 31, 72, 74, 75, 76, 77, 78, 79]. During attachment, the animals maintain their grip by friction with structures at micro- and nanoscale, i.e. papillae or microvilli [11, 14, 15, 16, 25, 28, 55, 56, 80, 81; see reviews 29, 30, 32]. Additionally, the mucus between and on these structures provides strong contribution to the attachment strength [82].

The whole body of the fish can be modified as ventral sucker (Balitoridae), or the fins have convergently evolved to attachment structures (Gobiesocidae, Cyclopteridae, Liparidae, Echeneidae, Cyprinidae) [18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 79].

In Gyrinocheilidae, some Gobiesocidae, and suckermouth catfishes of Africa (Mochokidae) and South America (Loricariidae, Astroblepidae), the mouth structures are transformed to an oral sucker, allowing animals to adhere to surfaces while simultaneously foraging and performing respiration [6, 7, 11, 12, 13, 20, 24, 74, 83]. This specialized suckermouth is, similar to attachment structures of other fish, highly textured with papillae bearing small keratinized outgrowths of single epithelia cells, i.e., unculi [10, 13, 14, 15, 16, 84, 85, 86].

In general, attachment structures in fish have received attention in the last decades, which even enabled the development of adhesive materials or gripping and adhesive devices [56, 69, 71, 78, 82, 87, 88, 89, 90, 91, 92; see reviews 29, 30, 31, 32, 93].

However, in contrast to remoras, gobies, hillstream loaches, darters, or clingfish, which were experimentally studied with regard to attachment performance and mechanisms [18, 25, 26, 55, 56, 62,
to date only few experiments [11, 72] addresses the real attachment performance of the loricariid suckermouth fish.

We here aim at presenting the structural diversity of papillae and unculi types in Loricariidae. As here only 69 species were studied, we expect that more diversity can be potentially discovered, when more species are included. As comparative experimental data on species with different mouthpart morphologies is lacking, we can only hypothesize about their attachment capabilities. As however, attachment structures were well investigated in various animal phyla and basic principles of adhesion and interlocking are known, we can infer some functionality based on the morphology analysis together with material property estimations and propose hypotheses about the interaction of papillae and unculi with different kinds of substrate (Fig. 4). This, however, needs to be proven experimentally in the future, especially with regard to the strength of current and substrate properties. In addition to the micro- and nanostructures, the mucus covering the mouth apparatus is on expect to contribute to the contact formation and adhesion as well and therefore should be investigated deeply in the future, in the course of controlled experiments on model species with different unculi and papilla types.

The loricariid oral disc is composed of upper and lower jaw and is surrounded by a softer outer rim, which was found to make tight contact with the substrates during attachment [6]. This seems similar to the soft tissues surrounding the suckers of remoras, which conform to the local roughness and curvature of the substrate [95], or to the outer papillae, setae or microvilli (which are of smaller diameter and densely packed) of the clingfish and loaches [25, 26, 55, 56, 80, 81].

The fleshy lips of Loricariidae are highly variable with regard to morphology, size and the content of collagen, which was previously found to relate to the substrate and the flow [96]. The collagen probably reinforces the oral suction cups and reduces slipping, failure or buckling in streams with high flow velocities [96], while being manipulated and bolstered by the jaws and maxillary barbels [12]. The lips are covered ventrally by uniculiferous papillae [9, 10, 12, 13], which probably increase wet friction and hydrodynamic adhesion to reinforce the seal of the oral disc [9, 11]. The geometry and arrangement of papillae in other fish taxa were previously found to support the resistance to shear forces and to arrests cracks at the interface between suction cups and substrate, which would compromise the subambient pressure in the mouth chamber [25, 26, 55, 80, 81]. This mechanism is partially similar to segmented adhesive pads of insects [41, 46, 97].

The unculi, which can be found on top of the papillae, are potentially involved in feeding [10, 12, 17]. But additionally, they probably increase the friction/interlocking during attachment on rough substrates [6, 11, 13, 14, 17]. This, together with the mucus, increases attachment strength as in other fish taxa [18, 82, 98].

With regard to interspecific variation of papilla size and morphology in Loricariidae, there are huge lacks of knowledge. In the here examined species, we were able to recognize four papillae types (Fig. 1), which differ in their height and range of motion, and eight unculi types (Fig. 2). With regard to the systematic position of the species (Fig. 3), it seems that there are high levels on convergent evolution, which was
previously also proposed for mandibles and body shapes in suckermouth armoured catfishes [2, 3]. High levels of convergences are also determined for foot adhesive pads in animals [46].

The flat papillae (detected in *Pseudacanthicus pitanga, Ancistrus ranunculus, Crossoloricaria cephalaspis, Pseudohemiodon almendarizi, Loricaria luciae, L. simillima, Spatuloricaria puganensis* and *Pterosturisoma microps*) seem to be composed of rather rigid material, which is embedded in the softer and more flexible lip (Fig. 4A). In some species (*A. ranunculus, C. cephalaspis, P. almendarizi, L. luciae* and *L. simillima*), no unculi were detected and the papilla surface seems to be rather bulky. During attachment, the papillae are probably capable of interlocking with rather the stiff and rough substrate (*A. ranunculus*), which is facilitated by the soft embedment. We, however, expect these species to underperform on stiff and smooth substrates and to hardly attach to soft substrates (plant covers), since contact areas are reduced (Fig. 4A). This could potentially explain, why this pattern is mostly found in species living on sand or mud (*C. cephalaspis, P. almendarizi, L. luciae* and *L. simillima*), where attachment will probably not play such an important role. Here, the species probably only temporarily attach to substrate (e.g., wood) and might not need a tight and more continuous attachment. However, thick mucus, covering the bulky surface, might compensate these shortcomings, which should be investigated in the future. However, in some species (*P. pitanga* and *S. puganensis*), these papillae are covered by flexible long and thin filaments or by small mushrooms. Here, we expect the unculi to adapt to the substrate increasing either adhesion (by filaments) or interlocking (by mushrooms) (Fig. 4E,G).

The short papillae were detected in *Hypoptopoma inexpectatum, Scobinancistrus aureatus, Ancistrus* sp. L464, *Chaetostoma formosae*, and *Hemiloricaria melini*. These papillae seem to have limited range of motion as well, but could potentially function as bolsters, when the unculi interact with the substrate, or support rearrangement during attachment, because the papillae tips seem to be flexible. They were either covered by unculi of the mushroom type (*S. aureatus, C. formosae*), hooks (A. sp. L464), or short mushrooms (*H. melini*). Here, unculi together with the flexible papillae could enable a tight interaction with the stiff and rough or with the soft substrate by interlocking and with the stiff and smooth one by adhesion (Fig. 4C,D,E). However, mucus could also be potentially distributed between the unculi and additionally support adhesion under water.

The medium sized papillae were detected in most studied species. Due to the length of the papillae we expect this type to have a higher range of motion, which presumably enables them to adapt to rather challenging surfaces. The bases of the papillae seem to be stiffer and the tips more flexible, and therefore we expect high attachment forces, as the flexible papilla tips (which make unculi bases flexible) can easily adapt to corrugated substrates and to interact with them. They were usually covered with unculi, either with mushrooms (*Otocinclus cocama, Ancistrus* sp. L519, A. sp. L107, *A. cirrhosus, A. luzia, Chaetostoma dorsale, C. lineopunctatum, Lamontichthys filamentosus, Sturisomatichthys aureus, Farlowella platyryynchus, F. oxyrryncha, Cteniloricaria platystoma*), hooks (*Panaqolus* sp. L271, *P. sp. L351, Panaque nigrolineatus, Scobinancistrus aff. pariolispos, Baryancistrus xanthellus* L81, L177, *B. aff. niveatus*), long filaments (*Pseudacanthicus spinosus, P. sp. L97, P. sp. L65, P. sp. L185, P. sp. L273, Sturisomatichthys festivus*) or short mushrooms (*Hypancistrus contradens,
*Hemiodontichthys acipenserinus*. The species bearing mushrooms, hooks, or short mushrooms can probably interact with the stiff and rough substrates and the soft substrate (plants, biofilm, etc.) by interlocking and with the stiff and smooth substrate by adhesion (Fig. 4C,D,E). However, on stiff and smooth substrates, contact points might be reduced, since less unculi are in contact. For the species bearing long filaments, we expect a high degree of interlocking on stiff and rough or soft substrates and of adhesion on stiff and smooth substrate, as these soft structures seem flexible enough to establish contact on most surfaces (Fig. 4G). The unculi of the fold type are potentially rather used for establishing contact by interlocking, since their tips seem to be rather stiff (Fig. 4H). We expect this type to underperform on soft substrates; however, mucus between these structures might increase their attachment ability. In one species (*Acanthicus adonis*), we determined medium papillae with a suction cups surface pattern. Due to morphology and material property estimation, we expect this type to adhere tightly with stiffer surfaces (both smooth and rough), but underperform on soft substrates (Fig. 4B), similar to the disc margins of clingfish (Ditsche et al., 2014). Only few species (*Rhinotocinclus isabelae, Leporacanthicus joselimai, L. sp. L240, Oligancistrus immaculatus, Parancistrus nudiventris, Hypancistrus* sp. L174, *H. zebra* and *Dekeyseria picta*) did not have unculi but rather a bulky surface (Fig. 4A). For these species, we expect an interlocking mechanism to be present. In this case, the relatively flexible papilla bases probably adapt to the target surface and the stiffer bulky surface enables interlocking with the rough and stiff substrate.

Long papillae were detected in some species (*Scobinancistrus* sp. L82, *Peckoltia* sp. L76, *Parancistrus aurantiacus, Hypancistrus* sp. L333, *Ancistrus claro, A. megalostomus, Aphanotorulus cf. emarginatus, Hypostomus bolivianus, H. laplatae, Pseudorinelepis cf. genibarbis* L95, L152, *Rhinelepis aspera*). Because of their length, we expect these papillae to adapt best to very rough surfaces, due to an increased range of motion. On top of these papillae, we detected either mushrooms (*S. sp. L82, A. claro, P. cf. genibarbis* L95, L152), folds (*P. sp. L76, A. cf. emarginatus, H. bolivianus, H. laplatae*), bulky surface without unculi (*P. aurantiacus*), long filaments (*H. sp. L333, *R. aspera*) or honey-combs (*A. megalostomus*). Since the mushrooms, long filaments and honey-combs seem to be flexible, we expect these structures to adhere to any surface either by interlocking or by adhesion (Fig. 4D,F,G). For the folds, which seem to be stiffer at their tips, we expect an underperformance on soft plant surfaces (Fig. 4H) due to their limited ability to adapt their shape to the target substrate, which would hinder the oral disc to form an effective seal, similar to gobies underperforming on rougher surfaces [22, 62]. The bulky surfaces (Fig. 4A) potentially also underperform on soft plant surfaces, due to the limited flexibility, and on stiff and smooth surfaces, because interlocking is here not facilitated. However, mucus might increase adhesion performance, which awaits further investigations in the future.

**Conclusion**

The oral discs of suckermouth armoured catfish (Loricariidae), which enable attachment and interlocking to various natural surfaces, are highly diverse with regard to the morphology of the papillae and of the unculi, small horny projections. Here, we studied 69 taxa and determined four papilla types and eight unculi types. From handling the structures and from drying artefacts we could infer some information...
about their material properties. This, together with their shape, enabled us to carefully propose hypotheses about mechanisms of interactions of oral disc structures with natural substrates typical for respective fish species.

**Methods**

**Specimens and preparation**

In this project, 69 species and undescribed taxa were studied (see Supplementary Table 1). For each species, between one and five specimens were examined, depending on the availability of material: some species are quite rare and only one specimen could be gathered, whereas for other taxa more individuals could be used. From the species with a higher quantity of individuals, we were able to study the intraspecific variability with regard to the morphology of the adhesive suckermouth papilla structures. Since this was not high, we decided to include the species with only one specimen in this study as well. Since papillae and unculi are regularly shed, we investigated every papilla on the studied lip part to obtain information about unculi types.

We did not experiment with living fish or kill fish for this study; instead we used the network of German suckermouth catfish owners (they were kept either directly by DKV or other hobbyists) which provided us with specimens that died naturally in the aquariums (fish died between 1995 and 2022). All animals were wild caught, imported to Germany by the ornamental fish trade, and then kept by hobbyists. Fish were, directly after death, fixed and stored in 70% EtOH.

For this study, we studied the lower left lip of the specimens, which were carefully dissected using scalpel and forceps. Samples were stored in 70% EtOH and cleaned from mucus by a short ultrasonic bath for 2 min. The samples were dried in a critical point dryer (Betta-Tech-Controls, Blakelands, UK). For this method, samples were transferred to 100% EtOH first, then to the liquid CO$_2$ and then slowly critical point dried from the CO$_2$.

**Scanning electron microscopy**

Lips were attached to scanning-electron-microscopy (SEM) sample holders by double-sided adhesive carbon tapes. Samples were then sputter-coated with gold-palladium (layer of 10 nm) employing a Leica EM SCD400 (Leica Microsystems, Wetzlar, DE). All samples were documented with a Scanning Electron TM3000 Tabletop Microscope (Hitachi, Tokyo, JP). All images were taken with different magnifications (30x – 2500x) at 5 kV.

Some lips from species with multiple animals at hand were investigated more detailedly employing a cryo-SEM S4800 (Hitachi, Tokyo, JP) equipped with the Gatan ALTO-2500 cryo-preparation system (Gatan, Abingdon, UK). For this purpose, the lips were carefully attached to SEM sample holders and then frozen in liquid nitrogen at -210°C, to avoid the formation of ice crystals, in the cryo-preparation chamber. Then,
the temperature was raised to -95°C, initiating the process of sublimation (freezing-drying). After sublimation for 5–7 min., the temperature was reduced to -140°C and the sample was sputter-coated by gold-palladium (layer of 3–4 nm) directly in the cryo-preparation chamber. Afterwards the samples were transferred into the SEM and observed at -120°C at 3 kV accelerating voltage.

We concentrated on two different hierarchical levels of the oral disc morphology: the labial papillae and the unculi on the papillae. In few species, the unculi type could not be determined due to high content of mucus. In these cases, we were only able to collect data on papilla morphology.

**Material property estimation**

We did not perform any material property tests for this manuscript. However, from the manipulation of papillae and unculi by forceps and from the observation of artefacts resulting from drying and shrinking, we can propose some hypotheses about the relative stiffness and flexibility of different sites of samples. We hope that the characterization of material properties of different parts of the sucker can be addressed by using micro- and nanoindentation in the future.

**Data on ecology**

Since data on the precise microhabitat of each species are lacking and await future investigations, we here rather chose rather broad categories (see Supplementary Table 1). The current was categorized in slow, medium and strong; the preferred substrate in wood, stone, wood and stone, or sand. This data was obtained from the personal observation of the collectors in the field (see Supplementary Fig. 1 and Supplementary Table 1).

**Systematization**

We studied 69 species belonging to four subfamilies of Loricariidae: Hypoptopomatinae, Loricariinae, Rhinelepinae and Hypostominae (see Supplementary Table 1). To gain insight, whether the specific morphology of the suckermouth structures relates to phylogeny, we plotted the data obtained on a cladogram. Here in this study, we did not use molecular information, but used recent phylogenies [34, 35, 36, 37] to place the genera accordingly. Within each genus, we sorted the species in alphabetical order, since there is, to the best of our knowledge, no phylogeny which includes all of the species studied.

**Statistical analyses**

As we here study nominal data, we used a contingency analysis to investigate the relationship between papillae, unculi, current, substrate and interaction type. This analysis was performed with JMP Pro, Version 14 (SAS Institute Inc., Cary, NC, 1989–2007).
Declarations

Ethics approval and consent to participate. Not applicable.

Consent for publication. Not applicable.

Availability of data and materials. The datasets are included in the Supplementary.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. DKV, and SG initiated the study. DKV, WK, and SG performed SEM analyses. DKV provided specimens and data on the ecology of species. WK collated the data and wrote the first draft of the manuscript. All authors contributed to and approved the final version of the manuscript for publication.

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**Figures**
Figure 1

Figure 2

Figure 3

Summary of ecological data and results from morphological analyses, visualized on a cladogram. From left to right: current type, preferred substrate, papilla type, unculi type, proposed type of interaction between substrate and organism – for each species studied. When the field is empty, no data was available.
Figure 4

Proposed interaction between some papillae and unculi types and the substrate (stiff & smooth, stiff & rough, soft). The grey color gradients in the structures relate to the mechanical properties (black = stiff; white = flexible), which are inferred from manipulation of structures and by documenting the artefacts caused by drying and shrinking. Purple boxes identify the structures, which probably facilitate interlocking (interlocking type) and the blue boxes identify the structures, which probably support adhesion to the target surface (adhesion type). Hexagons show, how presumably well the specific structures enable attachment with the target surface (orange = not well, yellow = medium, green = well).


Supplementary Files

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- SupplementaryMaterial.docx