

Dirt-sifting devilfish: winnowing in the geophagine cichlid *Satanoperca daemon* and evolutionary implications

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Abstract Winnowing is a foraging strategy common in geophagine cichlids (Cichlidae), in which sediment is sifted for food in the oral cavity. Geophagines have modified pharyngeal structures that function in winnowing, although detailed anatomical and functional information is still needed to clarify the mechanisms by which these fishes obtain food by sifting. With this study, we explore geophagine winnowing kinematics and variability of winnowing phases to test whether this is a highly modulated or stereotyped behavior. Winnowing was characterized with high-speed video of an archetypal winnower, *Satanoperca daemon*, which employs a three-part feeding behavior involving strike, winnowing, and sediment ejection. Over the course of feeding events, fish exhibited rapid reversal of hydraulic flow within the oral cavity and remarkable versatility during the winnowing stage. We also explored how cranial morphology varies within the clade across a phylogenetic hypothesis for the group. Cranial morphologies were analyzed in 19 species across 12 geophagine genera; principal component analysis suggests a particular winnowing morphospace exploited differentially by species. Central conclusions of this study are that the strike and ejection phases are stereotypic (low variation) but that rhythmic winnowing is highly variable. Winnowing in geophagines is not directly analogous to winnowing in

surfperches (Embiotocidae), to which it is often compared. There is substantial morphometric variation across the clade, even between winnowing species. The wide range of anatomical and biomechanical variants in this diverse clade provides intriguing insight into an underexplored feeding strategy.

Keywords Neotropical cichlids · Feeding kinematics · Substrate sifting · Morphometrics

Introduction

Cichlids represent one of the largest groups of Neotropical fishes, with more than 60 genera and nearly 600 species in South and Central America and Mexico (López-Fernández et al. 2010; McMahan et al. 2013). Among Neotropical cichlids, geophagines (literally, “earth eaters”) are named for a substrate winnowing feeding strategy common to many members of the clade, during which mouthfuls of fine-grained substrate are separated from edible detritus and microinvertebrates through oral manipulation. Most geophagines forage in sandy and silty substrates with abundant decaying plant matter (Kullander and Ferreira 1988). This foraging method is exhibited to some degree by most geophagines with few exceptions (most notably the *Crenicichla*–*Teleocichla* clade) (Arbour and López-Fernández 2013; López-Fernández and Taphorn 2004), but among these there may be great variability in how often and to what degree different species rely on substrate feeding as a contribution to overall diet (López-Fernández et al. 2014). Relative to other cichlid feeding strategies such as suction or ram feeding (Wainwright et al. 2001), there are few studies characterizing winnowing behaviors in live fishes. Winnowing in surfperches (Embiotocidae)

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was characterized by Drucker and Jensen (1991) using high-speed video analysis and has been noted in ecological and ontogenetic contexts in several marine percomorphs (McCormick 1998; Mueller et al. 1994; Schmitt and Holbrook 1984), but the behavior is much more widespread in the geophagines, whereas it is apparently restricted to only one or a few species in these clades. Foraging efficiency in different geophagines has also been studied more recently (López-Fernández et al. 2014), investigating whether certain species forage food optimally at different substrate depths. Although substrate sifting in the geophagines is usually assumed to be similar to that of the related embiotocids (López-Fernández et al. 2014), which use the pharyngeal jaws coupled with bidirectional water flow to mechanically manipulate a prey-debris bolus, this assumption has not been tested, and the quantitative kinematics and biomechanics of substrate sifting have never been formally characterized in geophagines.

Previous morphological studies identified what appears to be a clear anatomical signal for those species believed to engage in frequent winnowing, including deep heads, ventrally oriented and highly protrusible mouths, relatively weak pharyngeal jaws, and highly modified gill arches (López-Fernández et al. 2012) (Fig. 1a). Many winnowing lineages also have a highly modified pharyngeal arch structure, including an epibranchial lobe (EBL) of unknown function, suggested to play a role in feeding or mouthbrooding (López-Fernández et al. 2012) (Fig. 1b). The functional role of this morphology is unclear, although the modifications may aid in suction feeding, increased bite force, and post-acquisition oral and pharyngeal manipulation (López-Fernández et al. 2014). This morphology is

taken to an extreme in *Satanoperca* and the polyphyletic *Geophagus*, the two genera that engage most frequently in winnowing (López-Fernández and Taphorn 2004; Kullander 2012; López-Fernández et al. 2012).

Variability in the feeding anatomy of other winnowing geophagines, however, suggests a relatively unexplored diversity of winnowing morphologies. Although there are clear trends toward a specific morphology for frequent sifters, many geophagines rely only partially on winnowing as a foraging method (López-Fernández et al. 2012), behaving more as omnivorous generalists than benthic detritivores (Montaña and Winemiller 2009; Kullander and Ferreira 2005), while frequent winnowers have much higher proportions of organic detritus in the diet. These “partial winnowers” could suggest an interesting trade-off with respect to ecomorphological winnowing specialization and generalist optimization, which can only be explored if the functional role of winnowing morphologies is more clearly established in the geophagines. Moreover, evidence for which species among the geophagines do or do not winnow is often insufficient, based on anecdotal observations or laboratory settings which may not offer appropriate conditions to induce this behavior, especially in species for whom it may be only a secondary foraging strategy (Kullander and Ferreira 2005; López-Fernández et al. 2014). Thus, winnowing is a relatively poorly characterized feeding strategy of somewhat ambiguous origins in the geophagines (Astudillo-Clavijo et al. 2015), which warrants exploration with regard to behavioral kinematics, functional morphology, and evolution.

In the present study, our aim was to do a detailed study of the behavioral profile of winnowing using quantitative

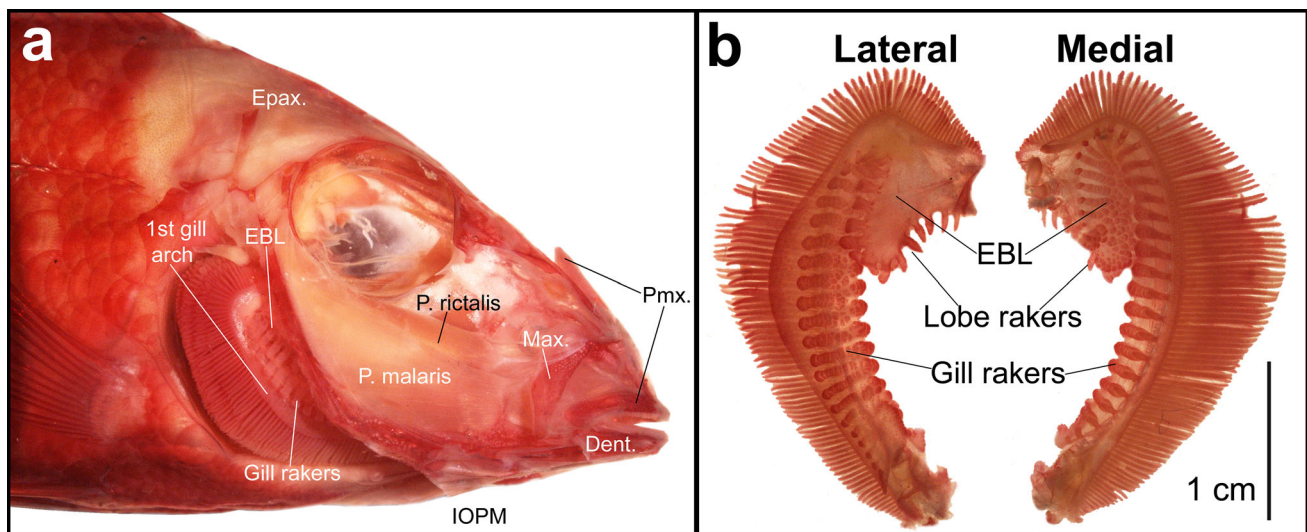


Fig. 1 Cranial anatomy and major gill arch components in *Satanoperca daemon*. **a** Superficial dissection of the *right side* of the head. Dent: dentary; Epax: epaxial musculature; IOPM: interopercular

mandibular ligament; Max: maxilla; P. malaris: pars malaris; P. rictalis: pars rictalis; Pmx.: premaxilla; **b** Lateral and medial views of the first gill arch of the specimen shown in **a** EBL epibranchial lobe

kinematics in an archetypal winnower, *Satanoperca daemon*, and a broader anatomical survey to establish functional morphological correlates of winnowing. How do earth eaters efficiently sift edible from inedible components of substrates? The first goal of this study was to characterize substrate sifting in *Satanoperca daemon* (Heckel, 1840), using high-speed video analysis to examine the strike kinematics and establish the behavioral phases of this feeding mode. How variable or stereotyped are winnowing behaviors? We set out to statistically test levels of kinematic variability (stereotypy vs modulation) of the various phases of winnowing. Which aspects of geophagine feeding morphology contribute most to this specialized foraging strategy? Our third goal was to examine how morphological diversity within geophagines varies with putative winnowing propensity using cranial geometric morphometrics of dissected specimens. This combination of approaches yields new insight into the winnowing feeding strategy of earth-eating cichlid fishes.

Materials and methods

Filming and video analysis

Feeding events were filmed in three wild-caught adult *Satanoperca daemon*, all between 9 and 13 cm SL, using high-speed video (Fig. 2). Fish were maintained in separate glass aquaria each with 2-cm pool sand (grain diameter 0.5–1.0 mm), with animal care and experimental methods approved under University of Chicago IACUC protocol 72365.

Fish were observed to winnow even in the absence of added food, but to induce frequent winnowing during filming a 3.5-ounce cube of frozen daphnia was thawed and added to the water. This typically resulted in a much higher number of winnowing events over a given time period. No distinction was made between feeding trials captured at different time points after food was added; it is possible that the normal grazing behavior (to which fish return 15–30 min after *Daphnia* addition) is not equivalent to the feeding behavior induced by the presence of high amounts of added food. Initial attempts to bury *Daphnia* did not result in qualitatively different feeding behavior, so the addition of food to the water column as a stimulant for winnowing behavior was employed.

Fish were filmed with a Photron FASTCAM SA7 at 500 frames s^{-1} . Video of substrate sifting was divided into three stages: strike, during which substrate is suctioned orally; winnowing, during which food and substrate are separated and substrate is ejected from the operculum; and ejection, during which larger particles are ejected orally. Strikes were defined as starting when the mouth first begins

to protrude and ending when oral gape first returns to a minimum after substrate has been suctioned (Fig. 4a–d). Winnowing was defined either as the time between the end of the strike and the start of ejection, or, if ejection does not occur, then the first point of minimum branchiostegal expansion after substrate is no longer being ejected from the operculum (Fig. 4e–h). Ejection was defined as starting from the first oral protrusion event after winnowing where substrate is ejected from the mouth, and continuing until oral gape returns to a minimum after the last oral protrusion (Fig. 4i–l).

Frames were digitized with the StereoMorph R package (Olsen and Westneat 2015) using a nine-point digitization protocol for the mouth, branchiostegal rays, eye, and epaxial musculature (Fig. 2). Because substrate suctioning often obscured view of the dentary while submerged in the sand, strikes were only digitized when view of the dentary was minimally obscured or not obscured. Every fifth frame was digitized for strike and ejection shots; the resulting trajectories were found to be equivalent to digitizing every frame in a shot. Similarly, every 15th frame was digitized for winnowing, because this stage was usually much longer than the strike or ejection stages, and this lower rate of sampling was sufficient to capture frequency and amplitude measurements accurately. For all videos, the area of the pupil was used as a scaling factor; eye area in pupils was found using MATLAB (2015). Distances between points of interest (branchiostegal expansion, premaxillary excursion, and oral gape) were computed in pixels and converted to millimeters using these scaling factors.

Cranial elevation was measured following the protocol of Westneat (1994), using the angle formed between the base of the dorsal fin, the anterior base of the pectoral fin (not shown in Fig. 2), and the tip of the maxilla. Cranial elevation just before, during, and after maximum oral gape was measured in a preliminary sample of five strike trials. Because measured angle change averaged only 1.55° with a standard deviation of 1.77° , cranial elevation was determined to be effectively zero and was not measured for the rest of the trials (Rice and Westneat 2005).

Analysis of kinematic variation

We used different statistical approaches to explore the three main phases of substrate sifting identified in this study. Because strikes and ejections were singular rather than cyclic events, stereotypy of these two stages was determined by taking pairwise autocorrelation coefficients between different trials for each of the oral gape, premaxillary excursion, and branchiostegal expansion measurements, after aligning by peak distance in each case. Autocorrelation coefficients were measured for two data-sets of each measurement: (1) areas of complete overlap

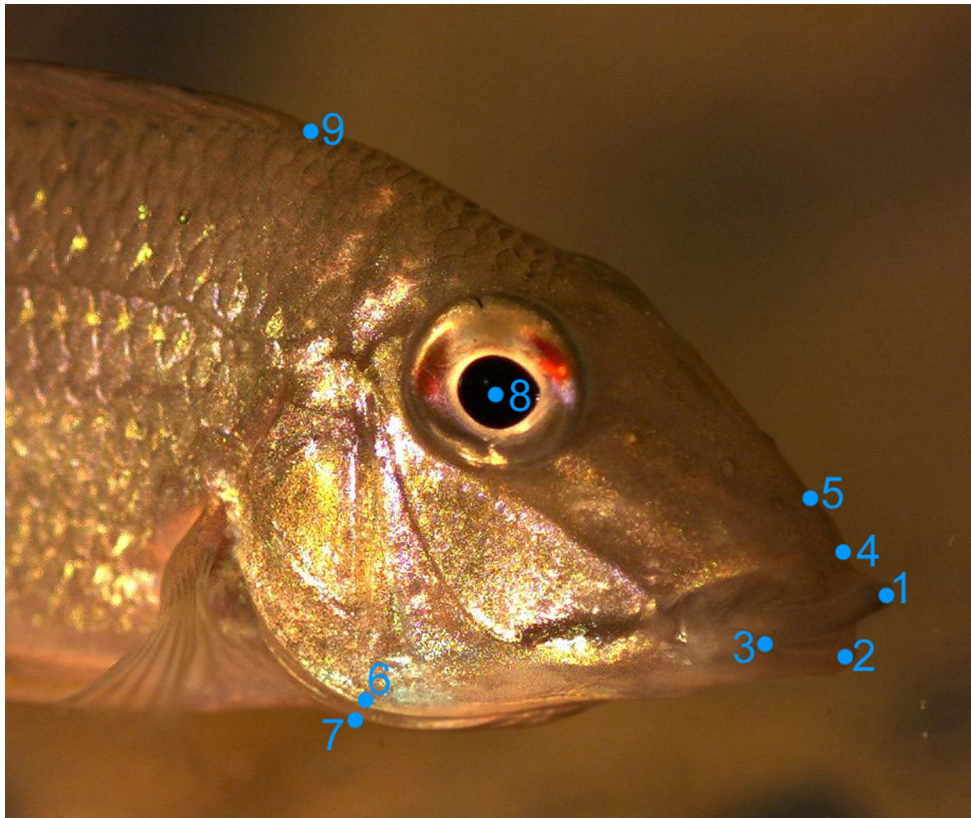


Fig. 2 Points digitized in every frame for video analysis. Nine points were used: 1 anterior tip of premaxilla; 2 anterior tip of dentary; 3 mouth corner, defined as the point where the upper and lower lips overlap; 4 anterior tip of the maxilla; 5 rotation point of the maxilla; 6

anteroventral tip of the operculum; 7 most distal branchiostegal ray outward from operculum point; 8 eye center; 9 base of first dorsal spine (proxy for epaxial musculature)

between all trials, thus restricting the autocorrelations to the length of the shortest trial; (2) areas of complete overlap between the ten longest trials, under the assumption that correlation would decrease when measured over a higher number of timepoints and that restricting to the length of the shortest trial could artificially increase the correlations. Higher autocorrelation coefficients correspond to more closely matched behaviors over time.

Correlation coefficient matrices were calculated using the autocorrelation function with lag 0 s in the R stats package. The Shapiro–Wilk normality test function from the same package was used to test whether the resulting coefficients were normally distributed, with the assumption that uncorrelated or highly variable behaviors would have correlation coefficient distributions centered around 0, and stereotyped behaviors would have a higher proportion of positive coefficients, indicating higher correlation than would be expected if the behaviors were not stereotyped.

Winnowing rhythmicity was determined by calculating the bias-adjusted coefficient of variation (CV) as a measure of spread for cycle duration, frequency, and number of cycles in every winnowing trial (Sokal and Braumann 1980):

$$CV = \left(1 + \frac{1}{4n}\right) \frac{s}{\bar{\mu}}$$

where n is the sample size and $s/\bar{\mu}$ is the uncorrected CV (standard deviation divided by sample mean). We tested for inter-individual variation using a simple GLM model for CV as calculated separately for each individual fish filmed using the GLM function in R and found that inter-individual variation was not significant ($p = 0.623$); therefore, we did not control for individual effects in winnowing variability.

Comparative morphology

Nineteen species across 12 genera representing the major geophagine clades were used from the collections of the Field Museum of Natural History and other research collections (Table 1). With the exception of *Biotodoma cupido*, at least two individuals from each species were dissected. Two or more species were chosen from the more speciose genera (*Crenicichla*, *Geophagus*, *Satanoperca*, *Apistogramma*, *Biotodoma*, and *Guianacara*), including three *Geophagus* due to the polyphyletic status of the

Table 1 Species and catalog numbers used in this study

Species	Catalog number(s)	SL (mm)
<i>Acarichthys heckelii</i> (Müller and Troschel, 1849)	FMNH 121822	66, 67
<i>Apistogramma cruzi</i> Kullander, 1986	FMNH 101577	48, 50
<i>Apistogramma rupununi</i> Fowler, 1914	FMNH 121835	54, 56
<i>Biotodoma cupido</i> (Heckel, 1840)	FMNH 55109	72
<i>Biotodoma wavrini</i> (Gosse, 1963)	FMNH 114449	64, 84
<i>Biotoecus dicentrarchus</i> Kullander, 1989	TCWC 11428.19	33, 35
<i>Biotoecus opercularis</i> (Steindachner, 1875)	MCP 30645	26, 27
<i>Crenicichla proteus</i> Cope, 1872	FMNH 101418	63, 64
<i>Crenicichla saxatilis</i> (Linnaeus, 1758)	FMNH 96098, 114466	71, 91
<i>Dicrossus filamentosus</i> (Ladiges, 1958)	AUM 43755	24, 31
“ <i>Geophagus</i> ” <i>brasiliensis</i> (Quoy & Gaimard, 1824)	FMNH 54194	55, 61
<i>Geophagus pellegrini</i> (Regan, 1912)	FMNH 58594	69, 92
<i>Geophagus surinamensis</i> (Bloch, 1791)	FMNH 117009	56, 65
<i>Guianacara geayi</i> (Pellegrin, 1902)	FMNH 110172	60, 66
<i>Guianacara owroewefi</i> Kullander & Nijssen, 1989	FMNH 117029	58, 65
<i>Gymnogeophagus gymnogenys</i> (Hensel, 1870)	FMNH 54267	61, 75
<i>Mikrogeophagus ramirezi</i> (Myers & Harry, 1948)	INHS 60430	24, 29
<i>Satanoperca daemon</i> (Heckel, 1840)	FMNH 114493	65, 72
<i>Satanoperca jurupari</i> (Heckel, 1840)	FMNH 101942	71, 75

Two individuals of each species were dissected, with the exception of *Biotodoma cupido*, for which only one was available. Individuals were taken from the same lot except in the case of *Crenicichla saxatilis*

genus. Dissections were limited to adults to avoid ontogenetic variation. Individuals were stained with Alizarin red in ethanol solution following the protocol of Datovo and Vari (2013). The right side of the head was dissected to reveal subdivisions of the adductor mandibulae complex and photographed using a Zeiss dissecting scope. Photographs of dissected specimens were digitized in ImageJ using the 19-point digitization protocol (Fig. 3a) established by Cooper and Westneat (2009). Principal component analysis was performed on Procrustes superimpositions of coordinates in both MorphoJ and R (Klingenberg, 2011; R Core Team 2016).

Results

Winnowing behaviors in *Satanoperca daemon*

Substrate sifting was clearly divisible into three distinct stages, performed successively: strike, winnowing, and ejection (Fig. 5). All three stages had characteristic associated trajectories with regard to oral gape, branchiostegal expansion, and premaxillary excursion events. Both the strike and ejection stages exhibited consistent patterns. Strikes always preceded other feeding stages and bore characteristics of typical suction feeding events in cichlids (Wainwright et al. 2001; Hulsey and García De León 2005).

During a strike, both oral gape and premaxillary excursion began to increase concurrently, peaking after 70–110 ms (Fig. 5a, b). The branchiostegal rays began to expand just as both oral gape and premaxillary excursion reached a maximum, peaking after 80–100 ms (Fig. 5c), suggesting unidirectional water flow from the mouth to the operculum, consistent with typical suction feeding. After peaking, oral gape quickly returned to a minimum, while both premaxillary distance and branchiostegal expansion decreased at much slower rates, meaning that the volume in the oral cavity remained higher. Strikes lasted an average of 0.33 ± 0.09 s (Table 2). Although cranial motion was tracked, no cranial elevation was observed, an intriguing result because cranial elevation occurs in the vast majority of suction feeding events for teleost fishes (Westneat and Wainwright 1989).

Winnowing typically commenced once the loose substrate/edible debris mixture entered the mouth. In order to effectively separate food and substrate, both the premaxilla and branchiostegal rays made brief, rapid expansions and contractions. Oral gape usually, but not always, cycled similarly (Fig. 5a–c, center panels), and typically at lower relative amplitude. During these excursions, substrate was ejected in bursts from the operculum, usually with a posteroventral trajectory (Fig. 4e–h). Opercular ejection coincided with local maxima of branchiostegal expansion. On one occasion, larger *Artemia* brine shrimp were fed to the two largest individuals

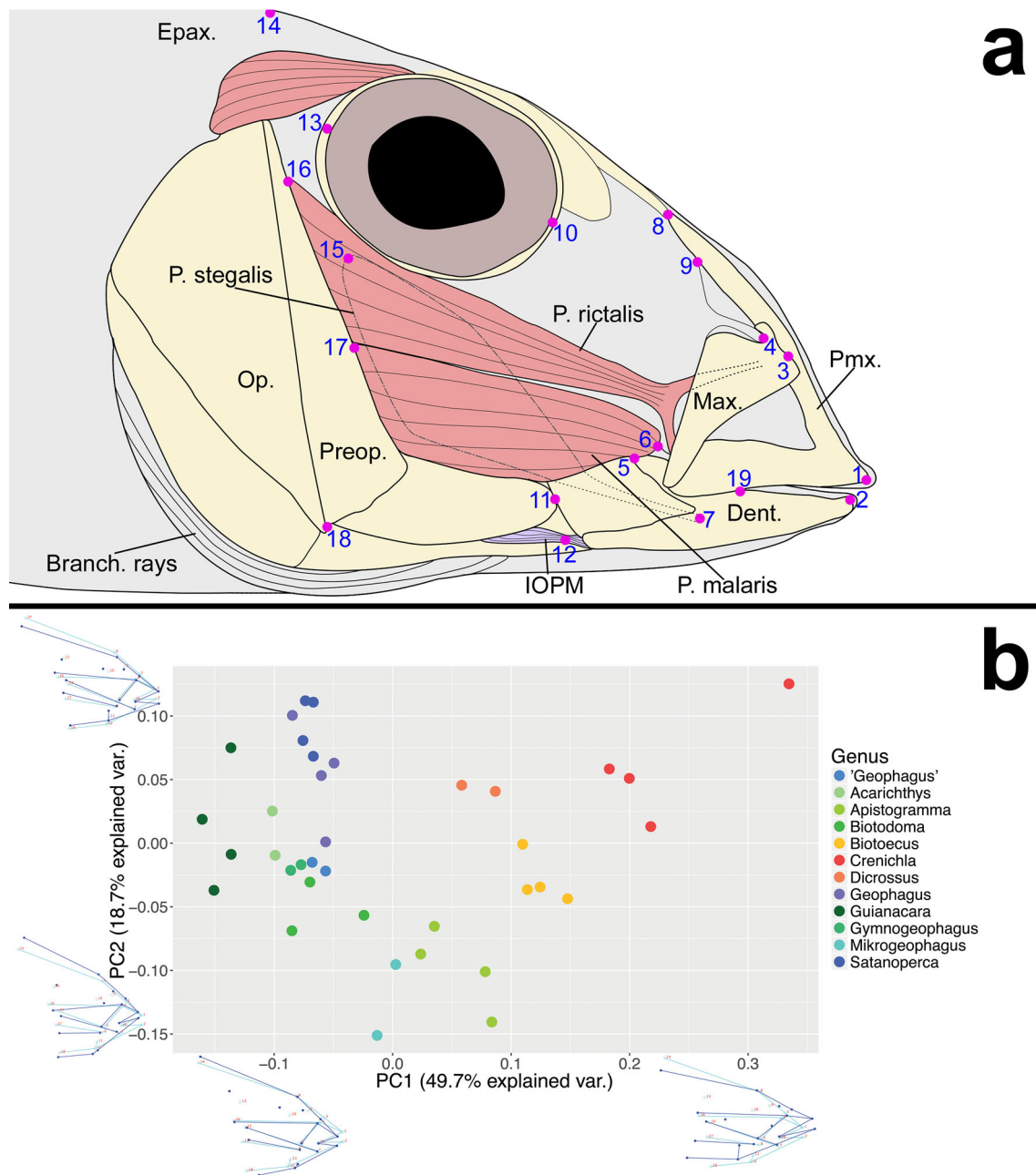


Fig. 3 Anatomical landmarks and resulting PCA. **a** (1) Tip of the anterior-most tooth of the premaxilla; (2) tip of the anterior-most tooth of the dentary; (3) upper rotation point of the maxilla (articulation with the palatine); (4) *pars rictalis* insertion on the maxilla; (5) intersection of the maxilla and articular bones; (6) *pars malaris* insertion on the articular process; (7) insertion of the *pars stegalis* on the medial surface of the articular; (8) tip of the ascending process of the premaxilla; (9) joint between the nasal bone and neurocranium; (10) anteroventral point of the eye socket; (11) joint of articular and quadrate (lower jaw joint); (12) midpoint of the

interopercular ligament; (13) posteroventral point of the eye socket; (14) tip of the supraoccipital crest; (15) most dorsal origin point of the *pars stegalis*; (16) most dorsal origin point of the *pars rictalis*; (17) most dorsal origin point of the *pars malaris*; (18) posteroventral corner of the preopercle; (19) mouth corner. **b** Principal components 1 and 2 for cranial diversity in geophagines. Putative winnowing species are in blue, purple, and green; orange, yellow, and red species are believed not to engage in winnowing. Warp wireframes for PC-scaled coordinates generated in MorphoJ are shown on their respective axes

used for filming; although both fish attempted to sift mouthfuls of substrate for these larger invertebrates, food of this size was almost always ejected with the substrate from the operculum during this stage.

During winnowing, fish cycled the substrate/debris mixture between 1 and 23 times, although the number of cycles was usually fewer than 10 (in 26 of 42 trials). Frequency varied between 4 and 8 Hz (Table 3). In general,

Table 2 Timing and gape during winnowing phases

	Strike	Winnowing	Ejection
Trials	28	41	32
Time (s)	0.33 ± 0.09 (0.27)	1.31 ± 0.93 (0.71)	0.53 ± 0.21 (0.40)
Oral gape (mm)	7.14 ± 0.99 (0.14)	3.63 ± 1.02 (0.28)	8.53 ± 0.85 (0.10)
Premaxillary protrusion (mm)	5.44 ± 0.70 (0.13)	3.46 ± 0.79 (0.23)	5.33 ± 0.80 (0.15)
Branchiostegal expansion (mm)	2.74 ± 0.55 (0.20)	2.53 ± 0.48 (0.19)	2.11 ± 0.63 (0.30)

Time for each phase is calculated using start and end points as defined in the text. Measurements are given as mean ± standard deviation, and coefficient of variation is given in parentheses. For each trial, maximum distance between each set of coordinates was found. The highest coefficient of variation for each measurement is bolded

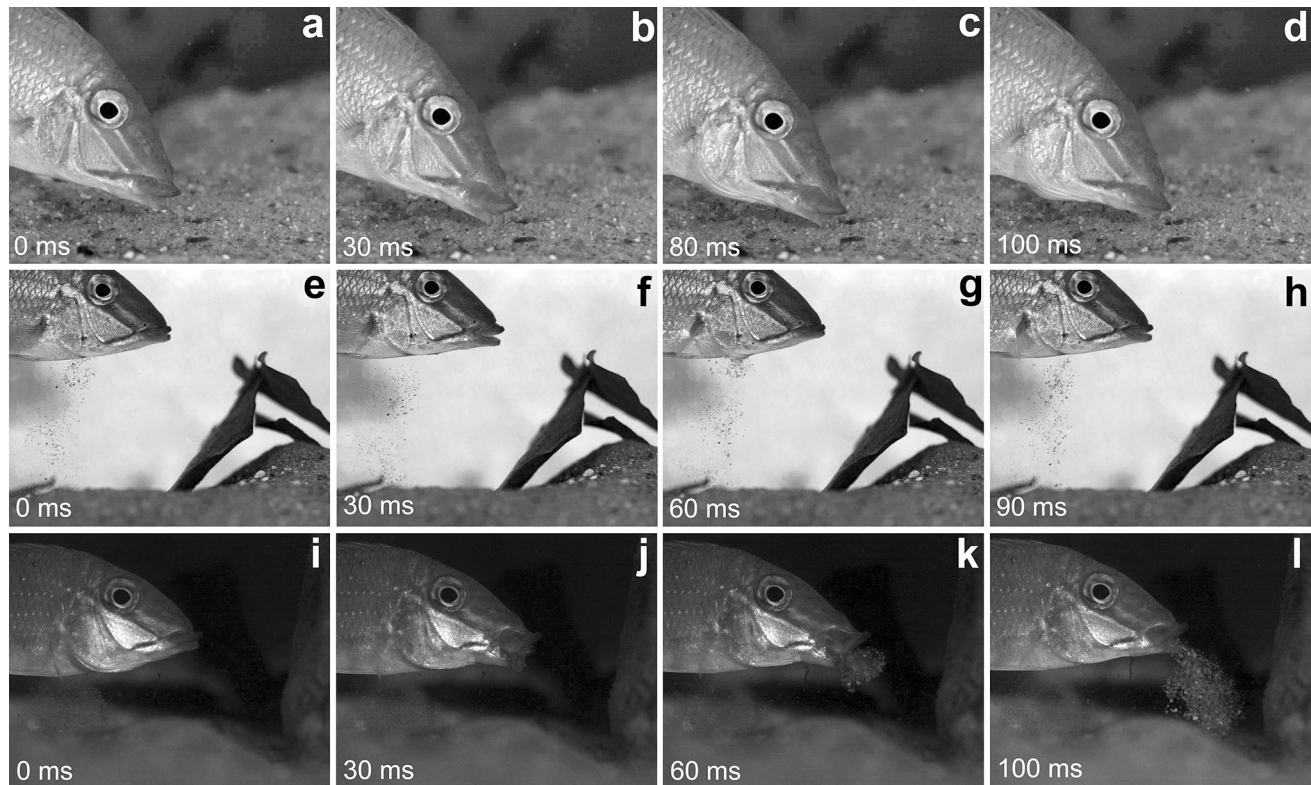


Fig. 4 Winnowing in *Satanoperca daemon*. Stills were taken from high-speed video trials. **a–d** Strike; **e–h** winnowing; **i–j** ejection. Elapsed time from the first frame is given in the *lower left-hand*

corner of each frame. Note bursts of sand being ejected from the operculum during winnowing, and lack of cranial elevation during substrate suction

Table 3 Variability during winnowing

	Average	Coeff. var.	Range
Frequency (s ⁻¹)	6.10 ± 0.99	0.204	4.17–8.33
No. cycles	7.77 ± 5.82	0.799	1–23
Cycle duration (s)	1.21 ± 0.75	0.625	0.33–3.34

Frequency is calculated as the average distance between local maxima for branchiostegal expansion, as it was found to have the greatest amplitude change in the three measurements. Cycle duration was determined by converting the number of frames in a video into seconds by dividing by 500 FPS

peak premaxillary excursion and peak oral gape occurred simultaneously during winnowing, while branchiostegal expansion typically lagged behind these peaks by about 25–65 ms (Fig. 5d). Compared to the strike and ejection stages, typical oral gape and premaxillary excursion had fairly low amplitudes, while branchiostegal expansion was similar in amplitude to other stages, and larger relative to mouth movements.

Ejection of debris usually occurred after winnowing, although in some cases fish engaged in several instances of

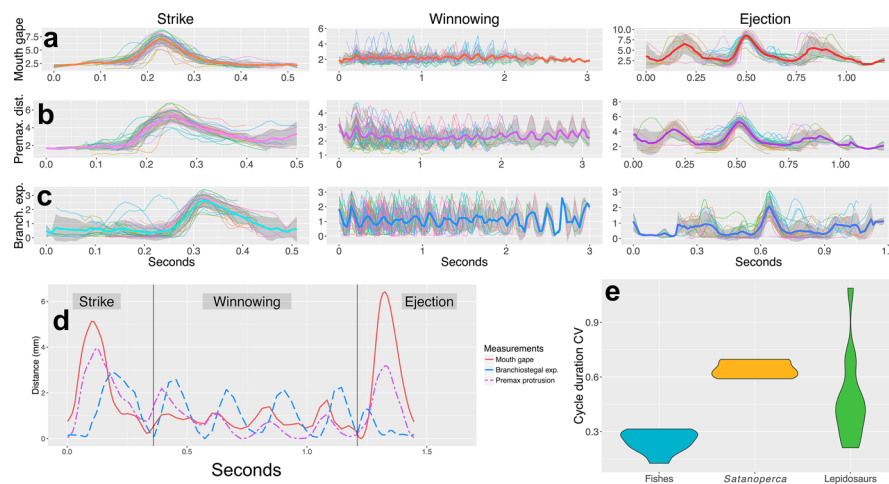


Fig. 5 Mouth gape, premaxillary excursion, and branchiostegal expansion during three distinct feeding phases of winnowing. **a** Mouth gape; **b** premaxillary excursion; **c** branchiostegal expansion; **d** all three measurements across the three feeding phases in a single representative trial; **e** distribution of coefficient of variation (CV) for cycle duration for nine basal teleost fish species, 13 lepidosaur

species, and three individual *Satanoperca daemon*. CV data for fishes and lepidosaurs taken from Gintof et al. (2010) and Ross et al. (2007). *Satanoperca daemon* CVs from this study. For **a–c**, trials were aligned by maxima and translucent gray bars indicate standard deviation

subsequent strike and winnowing phases before ejection occurred. During ejection, any particulates too large to be passed through the operculum were instead ejected orally forward, with some force, by reversing water flow from the opercular opening to the mouth. The behavior bears some resemblance to the buccal coughing exhibited by most teleosts for gill ventilation (Hughes 1960), in which water flow in the oral cavity is similarly reversed across the gills. There were typically between one and three ejections following a period of winnowing. Similar to strikes, both oral gape and premaxillary excursion (oral protrusion) increased together during ejections, although premaxillary excursion began slightly earlier (Fig. 5a–c, rightmost panels). Both peaks typically lasted between 65 and 90 ms.

Maximum branchiostegal expansion during ejection was typically lower than for winnowing or striking phases, with larger variation between trials (Table 1; Fig. 5e, f). Since water must be drawn into the buccal cavity before being directed orally for substrate ejection, it is possible water was suctioned through either the mouth or the operculum before ejection began. Branchiostegal expansion also occurred over a shorter period than oral and premaxillary expansion during this phase.

Highest overall oral gape was usually achieved during ejection (8.53 mm average, as opposed to 7.14 and 3.63 mm for strike and winnowing, respectively), while premaxillary excursions were greatest by a small margin during strikes. Branchiostegal expansion was more or less comparable across all three stages.

Stereotypy and variability in feeding stages

The pairwise autocorrelation coefficients for oral gape, premaxillary excursion, and branchiostegal expansion measurements during strikes and ejections show that these two behaviors show low variation, indicating that they are stereotyped. Restricting to the length of only the shortest trial (maximal overlap after aligning by peak), both strikes and ejections had strongly positive autocorrelation coefficients. Strikes were marginally more stereotyped (mean R of +0.603) than ejections (mean +0.560). To correct for artificially high autocorrelation due to length restrictions, the same test was performed on only the ten longest trials for both feeding stages, with the assumption that including more time points would lead to higher variability and lower average correlation. Strikes experienced the bigger drop in average autocorrelation coefficient (mean +0.386), while that of ejections remained virtually unchanged (+0.530). In each case, we determined that the distribution of autocorrelation coefficients was strongly non-normal using the Shapiro–Wilk normality test ($p < 10^{-5}$ for all tests conducted), with a left-tailed distribution centered between +0.6 and +0.9. Winnowing rhythmicity was determined by finding the CV of winnowing cycle duration, number of cycles, and frequencies across all complete filmed instances of winnowing (Table 2; Fig. 5e). Frequency had the lowest CV (0.204), while cycle duration and cycle number had much higher CV (0.625 and 0.799, respectively).

Feeding morphologies in geophagines

Consistent with previous morphological analyses, most winnowing species examined in this study had deep, triangular heads with narrow, ventrally oriented mouths (López-Fernández et al. 2014). Several other features of muscle morphology strongly associated with winnowing, including an increased degree of separation between the *pars rictalis* and *pars malaris* subdivisions of the adductor mandibulae, a proportionally larger *pars malaris*, and a

more posterior muscle–tendon transition in the *pars rictalis*, leaving more of the tendon exposed (Fig. 6d, e).

Even among the more frequent winnowers, however, there were notable differences in these more extreme morphologies. In both species of true *Geophagus* examined (*G. pellegrini* and *G. surinamensis*), fishes had distinct separations between the *pars rictalis* and *pars malaris* subdivisions, which were of approximately equal size, and in several cases specimens even appeared to have gaps between the two subdivisions (Fig. 6d). By contrast, both

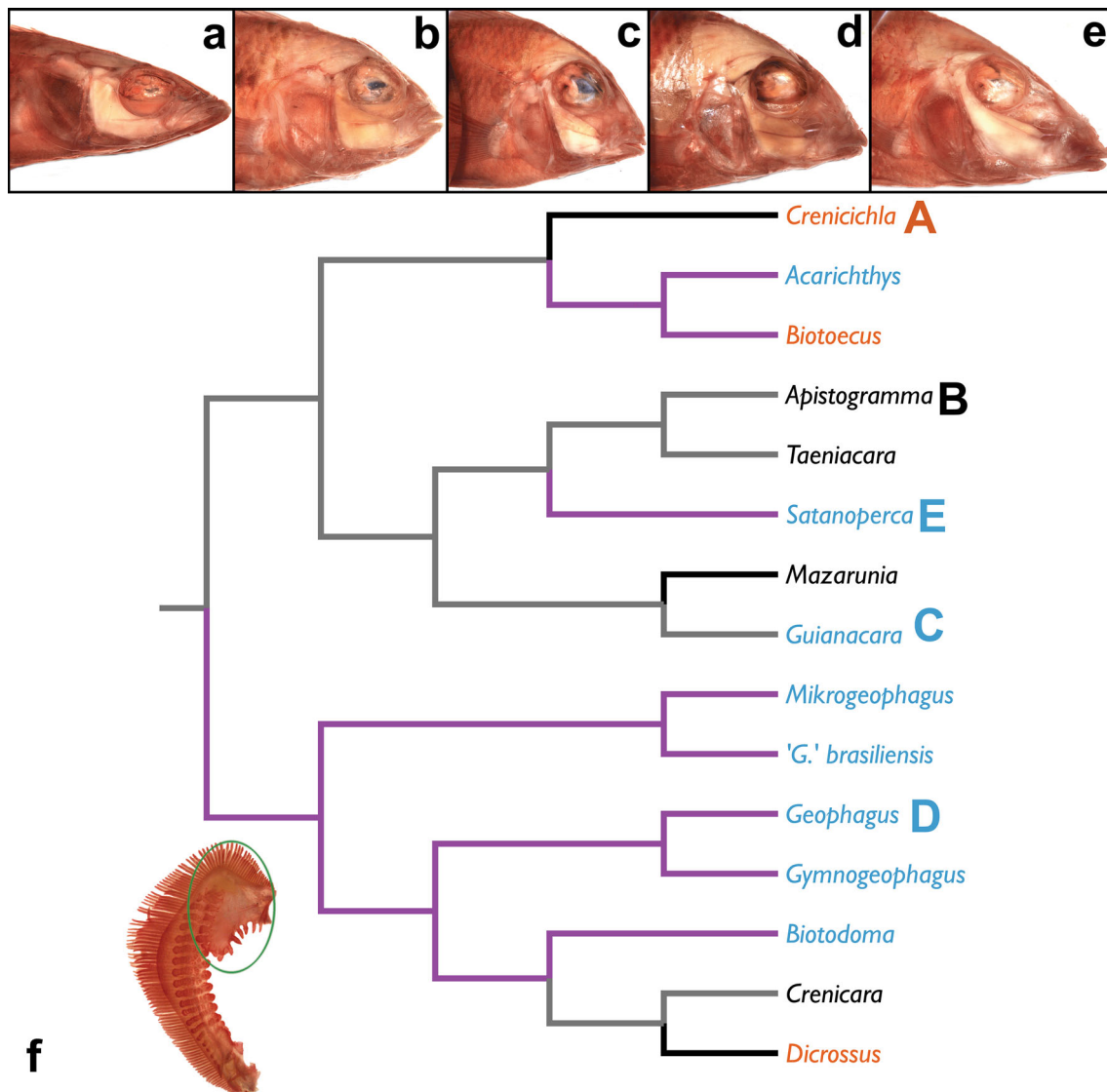


Fig. 6 Geophagine relationships and morphotypes. **a–e** Representative muscle morphologies of five geophagine cichlids. **a** *Crenicichla saxatilis*; **b** *Apistogramma cruzi*; **c** *Guianacara geayi*; **d** *Geophagus pellegrini*; **e** *Satanoperca daemon*. **f** Tree based on molecular data from López-Fernández et al. (2005). Taxon names are colored according to winnowing propensity; *blue* putative winnowing species; *orange* non-winnowing species; *black* either unknown or (in the case of *Apistogramma*) possibly not a genus-level character. Branches are colored *purple* according to the presence or absence of

an epibranchial lobe in the genus, *black* if EBL status in the genus is absent, and *gray* if EBL status or ancestral state is either unknown or ambiguous. Dissected heads and epibranchial of pharyngeal arches of the five specimens in **a–e** are indicated by *letter* in their respective locations on the tree and colored by the same scheme as taxon name. Ancestral states were estimated using maximum parsimony analysis, treating the EBL as a binary character. *Inset* shows the pharyngeal arch of *Satanoperca daemon* with the EBL circled in *green*

species of *Satanoperca* examined (*S. daemon* and *S. jurupari*) had a considerably larger *pars malaris* than *pars rictalis*, with by far the most dorsal origin on the margin of the operculum of any geophagine dissected. While these two most lateral subdivisions were clearly separate, the degree of separation was not especially extreme (Fig. 6e) or comparable to the separation observed in *Geophagus*.

Winnowing species that exhibited less extreme versions of these morphologies also displayed some of these characters to varying degrees. Like *Geophagus*, specimens of *Acarichthys*, “*G.*” *brasiliensis*, and *Guianacara* exhibited complete separation of the *pars rictalis* and *pars malaris*, with subdivisions of approximately equal size. *Apistogramma cruzi* (but not *A. rupununi*) also exhibited this distinct subdivision separation, although the status of winnowing in the highly speciose *Apistogramma* genus is poorly established (Kullander and Ferreira 2005).

Principal component analysis of 19 points in the head showed winnowing morphospace as being differentially exploited by winnowing geophagines (Fig. 3). PC1 (49.7% explained variance) varied mainly in mouth size and placement, head shape, and *pars malaris* insertion point. Negative PC1 values correspond to a smaller, more ventrally oriented mouth, a deeper head, and a higher *pars malaris* origin on the operculum, while positive values correspond to a longer, shorter head with a much larger, terminal or dorsally oriented mouth and lower *pars malaris* origin on the operculum. PC2 explains a much lower portion of variance (18.7%) and corresponded primarily to changes in mouth orientation, robustness of the lower jaw, and the angle of insertion of the adductor mandibulae.

Frequent winnowers (*Satanoperca* and true *Geophagus*) clustered over a fairly narrow range of PC1, as did other putative winnowing species including *Biotodoma*, *Acarichthys*, and *Gymnogeophagus*. Definite non-winnowers had exclusively positive PC1 scores. *Guianacara* specimens had the lowest PC1 scores. The smaller *Mikrogeophagus* and *Apistogramma* species were somewhat loosely grouped around intermediate PC1 scores between 0 and 0.1, in between the extremes of the frequent winnowers and *Crenicichla* ambush predators. While all putative winnowing species were quite tightly clustered on PC1, these species were spread much more widely across PC2. The frequent winnowers had some of the highest PC2 scores, while the less frequent winnowers scored much lower (“*G.*” *brasiliensis*, *Biotodoma*, *Acarichthys*, *Guianacara*, and *Gymnogeophagus*). *Mikrogeophagus* and *Apistogramma* species had the lowest overall PC2 scores, while the non-winnowers scored positively.

The tight clustering of the frequent winnowers across both PC1 and PC2 corresponded to a longer, more triangular head; a narrow, ventrally positioned terminal mouth; a more dorsal origin of the *pars malaris* and proportionally

smaller *pars rictalis*; and a relatively thin, flat lower jaw. The putative infrequent winnowers with negative PC1 and PC2 scores had more robust heads with even smaller mouths, although the mouths were more terminal rather than ventrally oriented; the dentary was also more rounded and robust than in the frequent winnowers. The non-winnowers, although variable, had more narrow, pointed heads, large, dorsally oriented terminal mouths, and a proportionally much larger *pars rictalis*.

Discussion

The results of the morphological and high-speed video analyses clearly indicate that winnowing is a diverse and complex behavior, which apparently correlates with a suite of morphological and behavioral traits that have non-uniform distributions among the winnowing geophagines. Characterization of the behavior in a frequent winnower, *Satanoperca daemon*, combined with morphological analyses, suggests that winnowing in this species is not directly analogous to a similar behavior in embiotocids; *Satanoperca* has an obviously different arrangement of the adductor mandibulae and typically sifts a more fine-grained substrate for much smaller microinvertebrates and particulate detritus. Although many of the individual feeding behaviors are fairly stereotyped in substrate sifting, these behaviors are combined modularly to produce high variability in the timing of winnowing events. While cranial morphometrics identify a clear suite of anatomical traits associated with a frequent winnowing morphology, the analysis suggests that geophagines have underexplored functional and probably behavioral differences that may have important ecomorphological implications. These morphologies may allow for particulate-level niche partitioning in substrate sifters, as specializing for efficient consumption of particular sizes or forms of microinvertebrate or detritus may come at the cost of being able to sort out other types of food in the substrate, but much more work is needed to determine whether this is the case. Among winnowers, species may sift for specific food types, and only some of these rely on winnowing as a primary foraging strategy. While the results of this study cannot provide complete resolution of ecomorphological trends in the geophagines, they clearly indicate an intriguing complexity in this unusual feeding strategy.

Substrate sifting in *Satanoperca daemon*

Characterization of superficial motion in *Satanoperca daemon* suggests control of bidirectional hydraulic transport in the oral cavity during feeding. Substrate is first

acquired in a fairly standard suction feeding motion, with rapid protrusion of the jaw causing water flow in the anterior–posterior direction. The strike motion may be stereotyped for all substrates (discussed below), but fish may also adjust this behavior depending on the type of substrate. For example, a silty substrate may require a smaller gape or slower strike than a sandy one, given the decreased particle size and ostensibly lower density.

Flow velocity changes rapidly during winnowing itself, although the direction remains largely in the anterior–posterior direction, as evidenced by the consistent lag of opercular expansion behind premaxillary excursion. The rapid cycling of oral and opercular expansions suggests that the magnitude of water flow itself is in near constant flux at this stage, peaking during oral expansion and decreasing as water is shunted out of the operculum. The frequency of cycling dictates the speed at which substrate is transported through the oral cavity, culminating in small bursts of loose substrate being ejected from the operculum (Fig. 4e–h). Whether or not fish take advantage of bidirectional water flow to manipulate the substrate–debris mixture with a kind of “hydraulic tongue,” as in the surfperches (Drucker and Jensen 1991), is unclear from these results.

Oral ejection necessarily involves reversing the typical direction of water flow through the oral cavity, in order to flush out larger particles that presumably cannot be filtered out at the gills. This behavior bears some resemblance to the gill-clearing buccal cough periodically exhibited by most teleost fishes (Hughes 1960) and performs a comparable function—any loose particles which have not been swallowed exit the mouth with some force. Exactly what triggers the transition from substrate sifting to oral ejection is unknown, since in addition to large particulates, sand alone is often ejected (Fig. 4i–l). Once edible material has been sufficiently separated from the substrate, fish indiscriminately eject remaining particulates, posing the question of how individuals determine the optimal switching point from winnowing to ejection.

While overall substrate sifting behaviors certainly indicate a superficial resemblance to surfperch winnowing with regard to orobuccal expansion and contraction and hydraulic transport, the behaviors are not as directly comparable, at least in the case of *Satanoperca daemon*. *Satanoperca* generally rakes a loose mixture of substrate and edible detritus, both fairly light, freely separate particles, and most of the inedible particles are flushed out of the oral cavity apparently without reaching the pharyngeal jaws, either through the ventral corner of the operculum or back out through the mouth; surfperches, by contrast, winnow primarily by manipulating a prey–debris bolus of much larger particulates (shrimp and macroalgae) in the pharyngeal jaws, relying on direct physical manipulation (Drucker and Jensen 1991).

Implications of stereotypy in feeding stages

Our results show that the three major components of *Satanoperca* feeding (strike, winnowing, and ejection) are variable in kinematics while also exhibiting fairly stereotypic patterns for strike and ejection, and rhythmicity similar to chewing in other vertebrates. The feeding behaviors associated with the strike and ejection behaviors are fairly stereotyped, as evidenced by high average autocorrelation between trials. Strike mechanics in *Satanoperca* are not especially variable as compared to other modes of suction feeding (Alfaro et al. 2001). When strike and ejection mechanics were restricted to regions of complete overlap between peak-aligned trials, stereotypy was comparable between the two feeding stages (0.603 and 0.560), suggesting the mechanics immediately surrounding peak gape are highly stereotyped in each case. Strikes virtually always had only one peak per trial (Fig. 5a–c, leftmost panels), indicating that fish would suction sand only once before winnowing it. Ejections, however, typically lasted much longer than strikes and often involved several instances of the flow-reversal events (Fig. 5a–c, rightmost panels). Although individual ejection cycles show evidence of stereotypy, fish evidently employ these cycles in variable combinations. While the feeding mechanics for both behaviors are similarly stereotyped, the actual deployment of these mechanics is therefore more variable during ejection.

The lower variability in strikes is appropriate given the fish's target: Sand or similar substrate is unlikely to offer the challenges posed by suction feeding on mobile prey in the water column, removing the need for modular acquisition. There is therefore not much source for mechanical variation and little need for variability during the strike stage. This stereotypy in initial substrate acquisition is supported by the lack of cranial elevation, suggesting that strikes are not especially forceful or speedy (Alfaro and Westneat 1999). The number of subsequent ejections following winnowing likely depends much more on the mouth contents, leading to the variability in the number and timing of ejection events after substrate is processed through winnowing.

The cycle duration CV of 0.625 is much higher than the average 0.248 established for nine species of basal teleost fishes by Gintof et al. (2010) using the same metric for chew cycle duration (Fig. 5e). Cycle duration CV was more similar to the lepidosaur average of 0.604 (Gintof et al. 2010). This variability in cycle duration can be attributed at least in part to the length and rhythmicity of this stage as compared to typical chewing events. In fish feeding, cycle variance typically increases with cycle time, with cycle durations that typically last between 300 and 600 ms; the average cycle duration for winnowing in

Satanoperca was much higher at over 1300 ms (Wainwright et al. 2008; Gintof et al. 2010). *Satanoperca* may have such high variation in cycle duration because there is so much variation in substrate mouthfuls—a patch of sand with almost no edible components may quickly be rejected resulting in a short cycle duration, while a patch of substrate with high concentrations of organic matter may be more carefully and lengthily sifted.

Possible parameters for determining the cycle duration of oral cycling include the amount of substrate initially acquired, the density and particle size of that substrate, the differences between edible and inedible components of the substrate (dictating the ease of their separation), or even the presence of unusual particulates. It was observed several times, for example, that fish attempting to winnow mouthfuls of substrate that included filamentous macroalgae would engage in highly irregular cycling, increasing and decreasing the frequency or attempting repeated ejections followed by regular processing, suggesting plasticity and responsiveness to variations in the mouth contents.

Interestingly, although winnowing seems to be a variable behavior with regard to length of cycle duration, fish engaged in some form of it regardless of whether or not inedible substrate was present in the mouth. Whenever loose food was suctioned from the water column, for example, the rapid premaxillary and branchiostegal expansions and contractions characteristic of substrate sifting were still observed, although no sand was present in the oral cavity. This potentially compulsive winnowing suggests either that this behavior is an automatic response regardless of whether it is needed for prey processing, or that it is required for successfully moving any food through the oral cavity and into the pharynx in addition to removing inedible substrate.

Winnowing mechanisms in geophagines

Feeding on microinvertebrates and detritus is by no means a rare diet among fishes, but isolating the edible from the inorganic components of a loose, fine-grained substrate can be exceptionally difficult from a mechanical perspective; unlike filter feeding in the water column, most of the solids that enter the oral cavity are inedible. Winnowers must separate two very similar components, both of which consist of small, loose particles. Separation of these components takes place in the oral cavity during winnowing, meaning that the repeated oscillations of oral and opercular expansion in combination with the cranial morphology allow the fish to swallow detritus and eject inorganic particulates.

One possible mechanism for substrate separation is that when the substrate/detritus mixture enters the mouth, the rapidly shifting water flow controlled by oral and opercular

expansion causes particulates of different densities to move at different speeds. Lighter material, including detritus and microinvertebrates, is more responsive to hydraulic transport than the substrate. With each successive increase and decrease (or reversal) of water flow, the lighter component remains suspended while the denser sand falls to the bottom of the oral cavity. The primarily anterior–posterior flow of water drives both the sand and detritus back toward the gills, at which point the sand is ejected through the operculum. The same motion which ejects sand from the ventral side, however, may cause the detritus suspension to encounter the EBL suspended from the roof of the mouth, so that rather than be ejected through the gills, edible components are raked into the pharynx to be swallowed.

Such a density-dependent mechanism would explain the muscle morphologies that are most strongly associated with frequent winnowing species. In *Satanoperca*, the *pars malaris* is much larger than the *pars rictalis* despite the head being fairly long and triangular, and not necessarily specialized for high bite force. A strong bite may not be especially advantageous for substrate sifters, given that substrate is usually both loosely packed and sedentary. Oral and pharyngeal manipulation, rather than substrate acquisition, places the most constraints on the feeding morphology. The *pars rictalis*, for example, is not only considerably smaller than the *pars malaris* in *Satanoperca*, but has a much more posteriorly shifted muscle–tendon transition, resulting in a noticeable space surrounding this tendon that in infrequent or non-winnowers is filled with muscle (Fig. 6, compare a–c to d–e). Given the premium placed on efficient use of surface area in the head (Hulsey et al. 2007), this space must have some functional role; it may allow for increased variability in oral manipulation, or even partially decoupled control of the mobile elements of the jaw. This would also explain the noticeable gap between subdivisions in the *Geophagus* species examined, assuming their mode of winnowing is comparable to that of *Satanoperca*. The large *pars malaris* in *Satanoperca* can be used to control periodicity of horizontal hydraulic transport in the oral cavity, moving water through in bursts so that lighter solids remain suspended, while inedible substrate has time to fall to the bottom of the mouth to be shunted out of the operculum. Notably, among the winnowing surfperches, morphology of the adductor mandibulae is essentially reversed relative to frequent winnowers in the geophagines we examined: The *pars rictalis* is by far the largest subdivision, with minimal division between the *pars malaris* and the *pars stegalis* (Drucker and Jensen 1991).

Geophagines may not rely on density and particle size alone to sift substrate; the EBL, rakers, and winnowing behaviors could serve only to agitate the mixture indiscriminately, and the EBL itself could be entirely

responsible for separating edible from inedible components. Our initial characterization of winnowing in *Satanoperca* did not vary substrate or food type, but variations of these parameters could provide further insight into the sifting mechanisms. Using a substrate of smaller grain size (such as silt or soil) or larger food, for example, could be much more difficult for *Satanoperca* to separate successfully, as supported by observations of their failure to feed on large particles. Varying the substrate and food types, even for a single species, might clarify exactly how winnowing morphologies allow geophagines to efficiently separate edible and inedible particulates. A more direct method would be to radiolabel food items and use X-ray imaging to visualize exactly how food masses are manipulated in the oral cavity, and if food is transported toward the EBL during winnowing (Gidmark et al. 2012).

Ecomorphological trade-offs in winnowing efficiency

The presence of multiple winnowing morphologies, as indicated by the variation in pharyngeal and muscle morphologies, implies that species are optimized to sift for different particles that are extremely difficult to separate from the substrate with more generic morphologies. Geophagines are usually classified as winnowers based on the amount of detritus or benthic prey in the diet (López-Fernández et al. 2014), including here (Fig. 6), but this classification alone is probably an oversimplification. There may be a size discriminant for substrate filtering in frequent winnowers; optimizing behavior and morphology for a particular kind of detritus or microinvertebrate may decrease efficiency in feeding on benthic food outside of this range, while dietary analyses would not differentiate these food types. Winnowing proficiency likely depends more on the physical properties of food items than their biological classification or their location on or in the substrate. Specialized winnowing morphologies for specific food types would allow for increased niche partitioning among geophagines.

The degree of specialization required for winnowing to be a primary feeding strategy requires extreme modifications that decrease the efficiency of more generic feeding modes. At least in *Satanoperca daemon*, there is an upper size limit on food items, as well as an apparently compulsory winnowing stage that negatively impacts prey-processing time when food is picked out of the water column. Given the difficulty of efficiently sorting the inedible from the abundant edible components of fine-grained substrate, however, this specialized morphology allows winnowers to exploit an abundant resource that is essentially inaccessible to other fishes of similar size. Among geophagines, most putative winnowing species do not have the extreme morphologies exemplified by

Satanoperca and *Geophagus* (Fig. 6). Many of these species (members of *Biotodoma*, *Acarichthys*, *Guianacara*, and *Gymnogeophagus*, among others) do have some morphological signal for winnowing supported by the presence of benthic material in the diet and limited behavioral studies (López-Fernández et al. 2014). These species probably do engage in substrate sifting, but in ways that differ from the substrate sifting exemplified by the *Satanoperca*–*Geophagus* cluster. Species in these genera may rely on winnowing much less, as a secondary or supplemental feeding strategy. Other species, including the morphologically unusual *Guianacara* genus, which had the extreme lowest PC1 scores, likely have a drastically different substrate sifting mechanism that requires a different set of anatomical traits. If so, it will be informative to examine exactly which winnowing characteristics species acquire and to what degree; the features that appear most frequently probably provide the greatest advantage for winnowing efficiency with minimal detriment to the species' primary feeding strategy. Similarly, the degree of importance that winnowing plays in the diet of a given species likely depends not only on the morphology, but on the specific habitat—substrate sifting in a region with low water flow and high sediment buildup is a much more viable strategy than substrate sifting in a turbulent environment with a substrate dominated by gravel or small rocks, for example.

Examinations of the relationship between feeding morphology and winnowing efficiency are limited by the lack of detailed behavioral data for feeding behaviors in geophagines. While substrate sifting morphologies are obvious in some winnowers, winnowing proficiency cannot be inferred from the morphology in less extreme cases. Of the two *Apistogramma* examined, for example, only one (*A. cruzi*) has an EBL, but otherwise no strong signal for winnowing based on PCA (Fig. 3b). Both species of *Guianacara*, conversely, group strongly with the winnowers despite entirely lacking an EBL. Behavioral information is crucial for understanding how these morphologies relate to geophagine feeding strategies, and what impact they may have on winnowing efficiency. Synthesizing information about morphology, diet, behavior, and common habitat will provide important ecomorphological insight into the role of winnowing in this large and diverse group of cichlids.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, under University of Chicago ACUP 72365. This article does not contain any studies with human participants performed by any of the authors.

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