

# Mechanics dictate where and how freshwater planarians fission

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**Abstract:** Asexual freshwater planarians reproduce by tearing themselves into two pieces by a process called binary fission. The resulting head and tail pieces regenerate within about a week forming two new worms. Understanding this process of ripping oneself into two parts poses a challenging biomechanical problem. Since planarians stop “doing it” at the slightest disturbance, this remained a centuries-old puzzle. We focus on *Dugesia japonica* fission and show that it proceeds in three stages: a local constriction (“waist formation”), pulsation - which increases waist longitudinal stresses - and transverse rupture. We developed a linear mechanical model with a planarian represented by a thin shell. The model fully captures the pulsation dynamics leading to rupture and reproduces empirical time scales and stresses. It asserts that fission execution is a mechanical process. Furthermore, we show that the location of waist formation, and thus fission, is determined by physical constraints. Together, our results demonstrate that where and how a planarian rips itself apart during asexual reproduction can be fully explained through biomechanics.

**Keywords:** planarians, biomechanics, fission, rupture, traction forces

**Significance Statement:** How planarians reproduce by ripping themselves into a head and a tail piece, which subsequently regenerate into two new worms, is a centuries-old biomechanics problem. Michael Faraday contemplated how this feat can be achieved in the 1800s, but it remained unanswered because it is experimentally difficult to observe planarians “doing it”. We recorded *Dugesia japonica* planarians in the act and developed a physical model which captures pivotal steps of their reproduction dynamics. The model reproduces experimental time scales and rupture stresses without fit parameters. The key to rupture is a local reduction of the animal’s cross-sectional area, which greatly amplifies the stresses exerted by the planarian’s musculature and enables rupture at substrate stresses in the Pa range.

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Already Michael Faraday and his contemporaries were intrigued by the observation that asexual freshwater planarians, squishy worms a few mm in length, reproduced by tearing themselves into a head and tail offspring, in a process called binary fission (1). How was it possible for these animals to generate the forces necessary to rip themselves using only their

own musculature and substrate traction? The question remained unanswered to this day, because it is experimentally difficult to study the fission process in sufficient detail to figure out how it works. Planarian fission is fast, violent, and irregular. No induction mechanism has been identified, although decapitation has been shown to increase fission probability (2–4). Furthermore, planarians are photophobic (5), fission occurs primarily in the dark (4, 6), and even slight disturbances cause it to stop, complicating real-time imaging of the process. Finally, in the planarian species most commonly used in stem cell research, fission occurs on average approximately once per month per worm (7) and only lasts from a few minutes to tens of minutes (this study). All these factors make fission dynamics hard to study and rendered it a neglected area of planarian research (8, 9), although fission and regeneration are intimately linked (2, 3).

The most comprehensive study of fission that we have found in the literature is the 1922 thesis (in French) of Vandel on asexual reproduction of several European *Dugesia* species (10). Vandel described fission as spontaneous and fast, varying in duration from seconds to minutes, and regulated but not triggered by environmental factors. He noted that the fission plane is highly variable along the head-tail axis. Furthermore, by observing two consecutive fissions of the same animal, the first occurring close to the head and the second almost at the center, Vandel concluded that it was “impossible to formulate rigorous conclusions. One must limit oneself to giving the general trends and looks of this phenomenon without trying to explain all the observed exceptions” [author translation] (10). Because where along the body axis a planarian divides affects the fitness and reproductive behaviors of its offspring (7, 11–13), understanding how fission location is regulated is an important question to be answered. Regarding the division process, Vandel described fission as a mechanical process, whereby the anterior and posterior parts act independently, with the anterior part rhythmically pulsing and the posterior part largely adhering to the substrate.

Here we focus on the biomechanics of fission in the asexual planarian *D. japonica*. Using time-lapse video recording, statistical analysis, and mathematical modelling, we show that Vandel was right in interpreting fission as a mechanical process, but wrong in declaring the fission location unpredictable. We dealt with the experimental challenges elaborated on above by decapitating specimen to increase fission frequency and recording events over the course of months to obtain data of the necessary quality for quantitative shape analysis. This imaging data was complemented by traction force experiments using special substrates, which were sufficiently soft and stable to allow for these kinds of long-term experiments.

The analysis of 22 fissions made it possible to identify three key stages shared among all events we observed in this species: a local constriction (“waist formation”), pulsation - which increases waist longitudinal stresses - and transverse rupture. As soft bodied animals, planarians exhibit these body shape changes through the action of perpendicularly oriented, antagonistic muscle groups on weakly compressible internal fluids and tissues, which make up what is called a hydrostatic skeleton (see reviews in (14, 15)). Waist formation is key to successful rupture, because it enhances the longitudinal stresses at a given longitudinal tension force exerted by the planarian’s musculature by an order of magnitude.

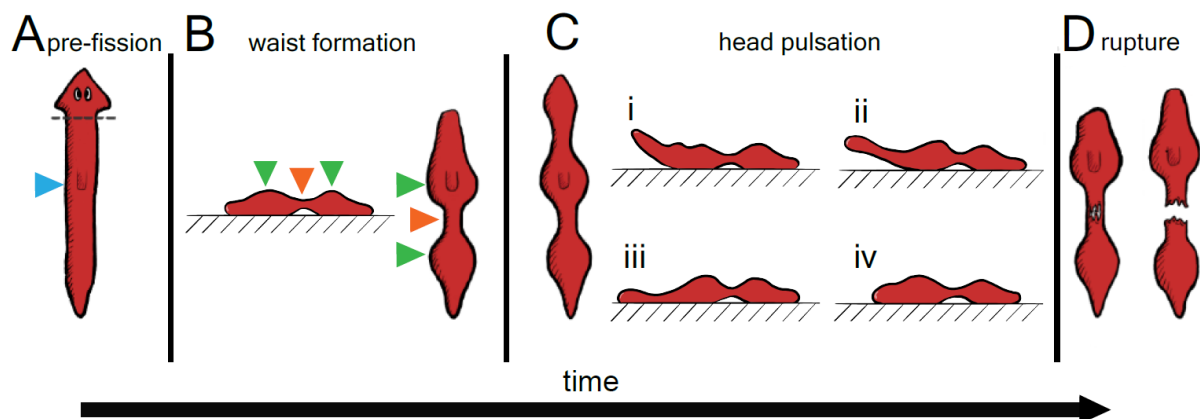
We found fission to be distinctively different from the three known gaits of planarian locomotion, which are gliding, peristalsis, and scrunching (16). Thus, fission poses a novel biomechanics scenario and the existing models that describe these planarian gaits are inadequate to describe fission dynamics.

Following D’Arcy W. Thompson’s thesis that “in the representation of form and in the comparisons of kindred forms (...) we discern the magnitude and the direction of the forces which have sufficed to convert the one form into the other” (17), we used the analysis of planarian body shapes to develop a linearized thin-cylindrical-shell model, which fully captures pulsation dynamics leading to rupture and reproduces empirical time scales and stresses. Importantly, the model only uses experimental data and parameters from the literature as inputs. This implies that rupture is a purely mechanical process that can be fully accounted for by physical mechanisms without requiring any additional biological explanations.

Besides solving this centuries-old mystery about the biomechanics of planarian reproduction, this study highlights the power of a practical approach, combining quantitative image analysis and a simple physical model, for gaining novel insights into a complex biological phenomenon which is not accessible to controlled experimentation and perturbations.

## Results

Months of continuous recording of decapitated *D. japonica* allowed us to capture a sufficient number of fission events occurring in open space for a quantitative study of fission dynamics. Decapitation promotes fission (2–4) without altering its dynamics (Movies S1 and S2), and was thus used as a means to increase the number of events. Qualitative analysis of these time lapse movies indicated that *D. japonica* fission occurs as a sequence of three distinct stages: waist formation, pulsation, and rupture (Fig.1 and Movie S2). Fission relies on the animal’s thin (10  $\mu\text{m}$ ) subepidermal muscle network (14), which consists of longitudinal (parallel to the head-tail axis), circular (perpendicular to the head-tail axis), and diagonal muscles (see also Fig.S1).



**Fig. 1. Cartoon of *D. japonica* fission. (A) Unperturbed planarian pre-fission. Pharynx is marked by the blue arrowhead. To increase fission rate (3), we amputate as indicated by the gray line. (B) Waist formation. Tissue movement causes local narrowing (orange arrowhead) and formation of wide contact regions at the head and tail (green arrowheads). Waist is not in**

**contact with surface. (C) Head lifts off substrate during pulsation and then re-adheres and slides back against the surface. (D) Rupture.**

Given the muscles' anatomical orientation, the vertico-lateral narrowing which leads to the formation of a waist is mediated by local contractions of circular muscles. Narrowing causes the waist region to lose contact with the substrate, while the body mass is actively redistributed toward the head and tail, leading to the formation of broad regions of contact with the substrate (Fig.1B).

Next, the pulsation stage starts, as the planarian lifts its head<sup>1</sup> from the substrate (Fig.1C), and "flesh waves," axially propagating lateral indentations of the worm head, are produced by contractions of circular muscle fibers. It appears that the generation of these waves is facilitated by the lack of contact (and, hence, of friction) between the lifted head and the substrate. As the planarian body is nearly incompressible (hydrostatic skeleton), these radial contractions produce longitudinal head extension and stresses in the waist. To return to its original shape the planarian then contracts its longitudinal muscles. Stresses in the waist are largest during the relaxation phase (contraction) of the head. When the longitudinal stress in the waist exceeds a critical value, rupture occurs (Fig.1D) and the worm flesh rips into head and tail pieces, concluding the fission. The two offspring regenerate into whole planarians in roughly a week.

As the cartoon (Fig.1) illustrates, fission dynamics are fairly complex. We first discuss where the waist forms. Then, we show how body shape analysis allows construction of a simple physical model that explains how pulsation can lead to rupture and estimate the magnitude of the rupture stresses.

***Location of Waist Formation.*** Vandel observed that an individual planarian divided at different locations when followed through consecutive fissions, which led him to the conclusion that the fission plane cannot be predicted. Because he did not study *D. japonica*, we checked whether this observation held equally true in this species. Indeed, as illustrated by the examples in Fig.2A, the fission location varies and it is seemingly impossible to predict where an individual planarian fissions (Note: the dynamics of these fission events were not recorded).

We then took advantage of a unique large-scale data set on the birth and division sizes, growth curves, and time between fissions ("reproductive waiting time" (RWT)) we had accumulated on *D. japonica* (18) and applied statistical analysis to assay whether those data would provide further insight. We found an asymmetric double-Gaussian distribution for the waist location based on imaging n=1335 specimen within 3 days after fission (see Fig.2B and Materials and Methods). Of note, the area of low fission probability between the peaks, as determined by manual inspection of a subpopulation of n=40 specimen, coincides with the location of the pharynx, which is a powerful muscle used to ingest food (19). Thus, planarians divide neither at a pole nor at the pharynx, but have a non-zero probability of dividing anywhere else along the head-tail axis, with the majority of events happening post-

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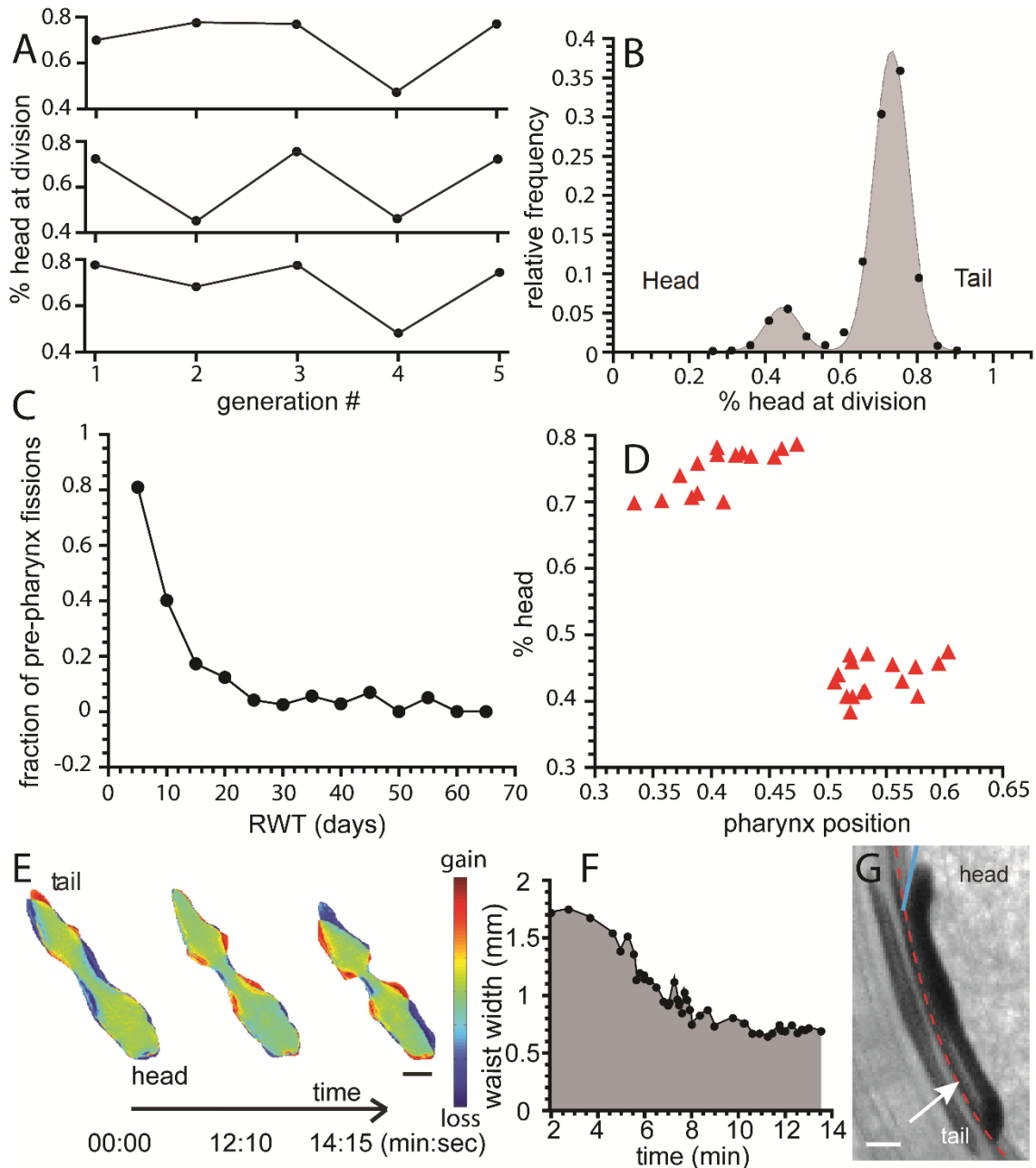
<sup>1</sup> Whenever we use the term "head", we refer to the region anterior to the waist. The true head was amputated in our experiments as indicated in Fig.1 A and described in the Methods.

pharyngeally. This distribution of waist position also explains why planarian fission is generally reported in the literature as occurring posterior to the pharynx (20–22).

Whether a planarian divides pre- or post-pharynx has a significant effect on its offspring, because it determines birth size and thus offspring survival and reproductive success (7). We therefore binarized the data in Fig.2B into pre- (%H <0.56) versus post-pharynx (%H >= 0.56) fissions and correlated it with other known quantities about the properties and history of these worms. While the following arguments hold true for all *D. japonica*, we only discuss individuals originating from a head offspring below, because pre-pharynx fissions are negligible for planarians that originate from tails (<< 1%).

We found a strong correlation between a planarian's RWT and fission location (Fig.2C), whereas the planarian's size at division had no effect (Fig.S2). Nearly all pre-pharynx fissions resulted from worms with short RWTs (< 2 weeks; Fig.2C). Upon inspection of the physical characteristics of these planarians, we found that we can predict where (either pre- or post-pharyngeally) an individual *D. japonica* will divide through quantification of its relative pharynx position (Fig.2D). Fission occurs anterior to the pharynx when the pharynx is located relatively closer to the tail (Fig.2D). This is frequently the case for animals with short RWTs (Fig.S2). A comparison of pharynx positions at birth and at division of pre- and post-pharyngeal dividers shows that the former have not repositioned their pharynx sufficiently to allow for a post-pharyngeal fission. Because repositioning takes time, this can explain why we primarily observe pre-pharynx divisions in rapid dividers.

We can explain why pharynx position matters with biomechanical arguments. To pull itself apart, a planarian needs to form two sufficiently large contact regions (adhesion patches) with the substrate. If the pharynx is located close to the tail end, the posterior part of the animal is too small to accommodate the adhesion patches and fission occurs anterior to the pharynx (Fig.S2). Because the size of these patches scales with worm size (Fig.S2), absolute worm size does not matter.



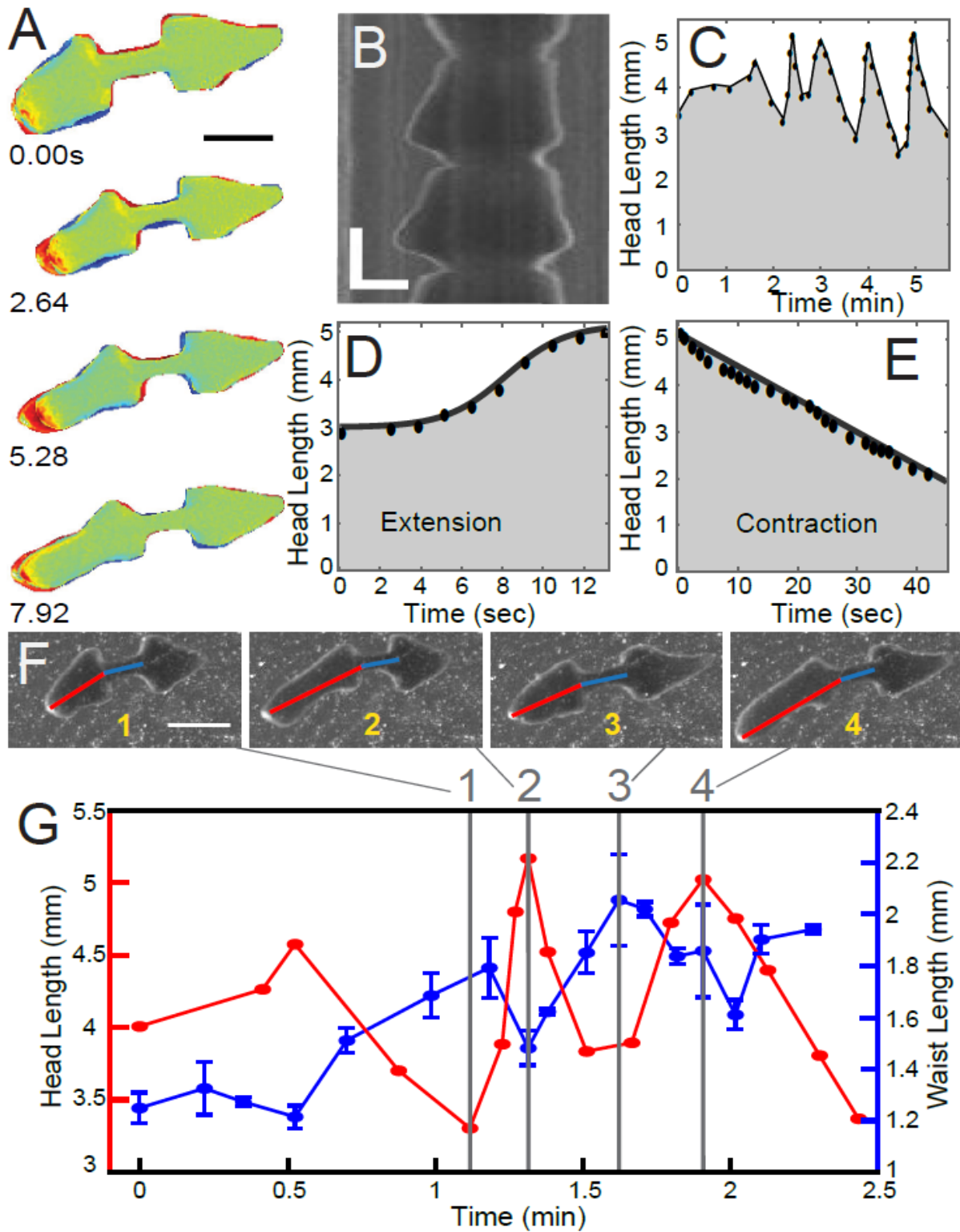
**Fig. 2. Waist formation.** (A) Waist position along the head-tail axis is not conserved across generations for a single planarian line. (B) Frequency of occurrence of waist position, expressed as % head at division (N=1335). (C) Quantification of the fraction of pre-pharynx fissions as a function of reproductive waiting time (RWT). (D) Quantification of % head at division as a function of relative pharynx position shows that the latter predicts fission position. (E) Color-coded maps of worm footprint, showing a representative sequence of waist formation in an amputated planarian. Colors indicate the change in the mass per unit area, with green corresponding to no change, blue - loss, and red – gain of mass. Scale bar=1mm. (F) The width of the waist region vs. time in panel E. (G) Side view image of a planarian undergoing fission. Dashed red line outlines Petri dish wall. The white arrow highlights a gap between the waist of the worm and the substrate. The blue line indicates the angle by which the planarian lifts its head prior to pulsation. Scale bar: 1mm.

To summarize, our statistical analysis shows that it is possible to predict whether a particular *D. japonica* planarian will fission pre-or post-pharynx, solely through quantification of its relative pharynx position.

**Mechanism of Waist Formation.** Waist formation, which is a local narrowing in the vertical and lateral directions, is achieved by contractions of circular muscles. Peristaltic contractions move mass from the waist region toward the head and tail (Fig.2E, F, Fig.S3A-B and Movie S3). As a result of this mass redistribution, the area of contact with the substrate on either side of the waist increases, whereas the waist part of the worm body detaches from the substrate (Fig.2G and Movie S4). Waist formation is critical for successful fission. It has the physiological benefit of preventing gut spillage during the subsequent rupture. Not less importantly, for a given longitudinal tension force, the reduction in the worm cross-section in the waist region leads to a proportional amplification of the longitudinal tensile stress. That said, the waist diameter is anatomically constrained and scales with the initial width of the worm (*Supporting Information* and Fig.S4). This scaling indicates the existence of a mechanism preventing the formation of waists that are too narrow. Experimentally, we found that the ratio of cross sections of the head,  $A_{head}$ , and of the waist,  $A_{waist}$ , is limited by  $\sim 14$ , suggesting that the tensile stress at the waist can be amplified by a factor of up to  $\sim 14$ , as compared with the characteristic traction stress in the head (or the tail) (see *Supporting Information* and Fig.S4).

**Pulsations.** Once the waist is formed, pulsations begin, *i.e.* the planarian executes multiple longitudinal extensions and contractions of the head and/or tail parts of its body (Fig.3 A-C, Fig.S3C-D, and Movies S5 and S6). On average, head pulses occur more frequently than tail pulses (89 head pulses versus 19 tail pulses total for  $n=22$  fissions) suggesting that head pulses are critical for generating tensile stresses causing the rupture in the waist region. Notably, head and tail pulses were asynchronous and we never observed a “tug-o-war” between them.

At the beginning of each head pulse, the anterior of the head detaches from the substrate (Fig.2G, Fig.S5, and Movies S4 and S7). The detachment allows the head to break out of the mucus layer (see *Supporting Information* and Fig.S5 and Fig.S6), minimizing friction with the substrate during head extension. Circular muscle contractions then elongate the head (Fig.3D and Fig.S3). This is a necessity of the planarian’s hydrostatic skeleton: If a worm elongates, while both its width and height decrease, it can only be a result of contraction of the circular muscles. The head reattaches to the substrate and slowly contracts. Head contraction is achieved by shortening of the worm’s longitudinal muscles and resisted by friction with the substrate (Fig.3E). These different dynamics are clearly seen in Fig.3(D-E), where head length during pulsation increases logarithmically (S-shape in Fig.3D), but decreases linearly with time. Importantly, head and waist dynamics are anticorrelated. As the head extends, the waist gets compressed and buckles (Movies S5 and S6). As the head contracts, the waist region gets stretched (Fig.3F, G).



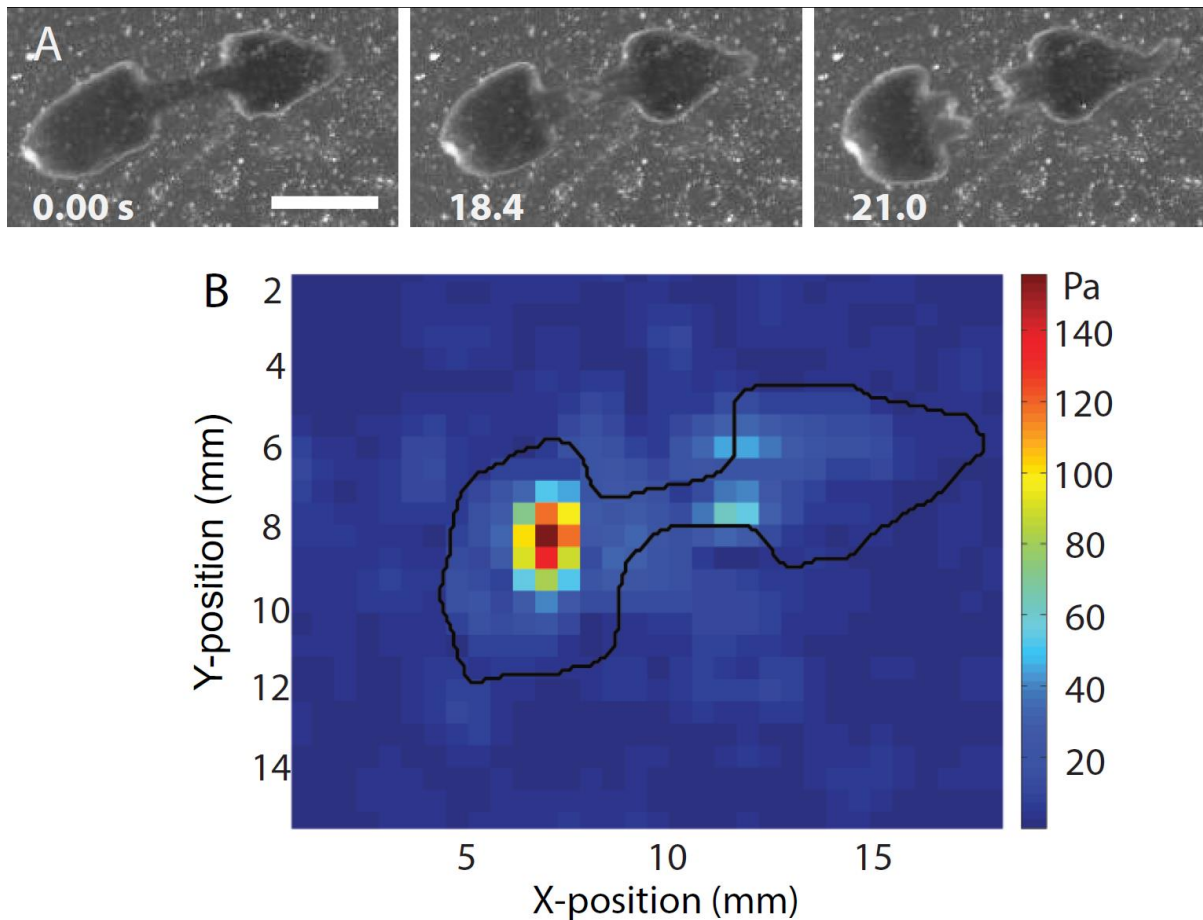
**Fig. 3. Pulsation.** (A) Representative sequence of head pulsation of an amputated planarian. Color-coding is the same as in Fig.2. Scale bar = 3mm. (B) Kymograph showing multiple head pulses, vertical scale bar = 20s, horizontal scale bar = 1 mm. (C). Head length as a function of time during a series of head pulsations. (D) Head extension is logistic and fast, whereas (E) head contraction is linear and slow. (F-G) Head and waist lengths are anticorrelated. (F) Consecutive images of a planarian with its head and waist lengths shown by red and blue lines, respectively.



**Scale bar =3mm (G) Time dependences of head length and waist length of the planarian from the images in (F).**

The maintenance of proper adhesion with the substrate is crucial during this stage. We observed some animals slipping during pulsation (Fig.S6) and interpret this as resulting from weak adhesion with the substrate, leading to poor stress transmission to the waist. In accordance with this interpretation, animals that slip execute more pulsations prior to rupture. Interestingly, the number of pulses was weakly anti-correlated with a planarian's size, suggesting that the absolute size matters for successful fission (Fig.S6). In other situations where substrate adhesion is critical, we and others have shown that an increase in mucus secretion is key (16, 23). To directly prove the link between a planarian's mucus secretion and adhesion, we treated planarians with TritonX-100, which increased mucus secretion (Fig. S6). Using a custom aspiration setup, we then quantified the aspiration force required to detach worms from their substrate. TritonX-100 exposed planarians required larger aspiration forces than control planarians (Fig. S6). Based on these data, we postulate that substrate adhesion during fission is mediated through the increased presence of mucus.

**Rupture.** The ultimate and key step in fission is successful rupture (Fig.4A). Rupture occurs when the stress in the waist, induced by contraction of the head, exceeds a critical threshold (Fig.S4E). In most cases (20/22 fissions, see Methods and Movie S8), rupture is nucleated at the center of the waist region. We measured the stresses exerted on the substrate during fission using traction force measurements (Fig.4B). To this end, we fabricated thick (~ 5 mm) soft (Young's modulus  $E = 1.2$  kPa) silicone gels substrates with 30-45  $\mu\text{m}$  diameter beads imbedded in an ~70  $\mu\text{m}$  thick surface layer as the tracer particles (see Methods). A map of displacements of the beads from their zero-stress locations (when the substrate was not deformed by the worm) was generated and converted into a map of traction stresses using previously published algorithms (24) (see Methods). The stresses were on the order of 100Pa (Fig.4B).



**Fig.4: Rupture. (A) Image sequence of a worm rupturing; scale bar = 2 mm. (B) Color-coded map of the substrate traction stresses produced by a *D. japonica* (contour in black) a short time before rupture.**

Projection of the stresses on the axis of the worm (direction along the waist) was then integrated over the areas of the head and of the tail, providing the pair of opposing traction forces that stretch the waist. The stretching force before rupture was a few hundred  $\mu\text{N}$  and, when divided by the cross-sectional area of the waist, it provided an estimate of  $\sim 2000$  Pa for the tensile stress in the waist immediately before rupture. Once rupture is completed, the two offspring move independently and regenerate into new full worms within about a week.

A comparison of the fission dynamics of events on soft PDMS gels versus plastic Petri dishes revealed no significant differences in terms of the number of pulses (Fig.S6), suggesting that the interaction with the mucus dominates substrate effects on fission.

It is evident from this quantitative analysis that fission dynamics have little in common with normal planarian locomotion via cilia based gliding, which does not involve body shape changes (16). There are some similarities between fission and the two muscle-based planarian gaits, peristalsis and scrunching (16, 23), insofar as all involve body elongation-contraction cycles and require good contact with the substrate for successful execution. However, fission pulsation dynamics are different from those observed in peristalsis or scrunching, which either show no asymmetry or relatively longer elongation periods, respectively. Finally, waist

formation is a unique feature of fission. Because of these differences, existing models for these gaits fail to reproduce the observed fission dynamics.

Therefore, we developed a new linear mechanical model with the planarian head represented by a thin cylindrical elastic shell (corresponding to the thin musculature network) filled with a viscous liquid (corresponding to coarse-grained squishy internal tissue). The same model could be applied to the tail part on the other side of the waist, but we focus on the head because tail pulsations do not occur in all fission events. Although the deformations during fission are large, this linear thin shell model allows us to capture pulsation and rupture dynamics using only physical arguments and scaling estimates.

**Physical Model.** We treat the head as a uniform, long, thin cylindrical elastic shell of cross-sectional radius  $R$ , Young's modulus  $E$ , and shell thickness  $h$  which encloses material of density  $\rho$  (Fig.S1). Although planarians, being flatworms, have elliptical cross-sectional areas, the simplification to circular cross-sectional areas has a negligible effect on the results (see *Supporting Information*). We assume that the head is connected to the waist by an impermeable junction, through which no matter crosses on the time scale of pulsations (Fig. S1). This assumption is reasonable, as the data shows no material transfer once the waist has been established (Movies S4 and S5). The long-thin approximation applies, since  $L_{head} \sim 4mm$  while  $R_{head} \sim 1.2mm$ .

A thin cylindrical elastic shell supports three modes of waves, namely longitudinal, flexural, and torsional. The last ones can be ignored as there is no indication that the planarian twists during pulsation. Longitudinal wave displacements on an elastic cylinder are predominantly axial while flexural (F)-waves support primarily radial (lateral) displacements. These two modes are linearly coupled at finite Poisson ratio (see eq. (1-3) in *Supporting Information*)).

The experimentally observed flesh waves correspond to F-waves. They are initiated by contractions of circular muscles in the head part anterior to the substrate contact region, causing local changes in the radius. The head anterior is detached from the substrate during extension and therefore has no friction with it. The deformation propagates longitudinally at the group velocity of F-waves. As the wave progresses anteriorly, the head extends forward due to volume conservation. Simultaneously, the wave propagates in the head posterior toward the waist region and because the head-waist connection is impermeable, the waist region experiences a compression and buckles. Thus, qualitatively, the model predicts the observed anticorrelation of head and waist length during head extension. Furthermore, we can compare the F-wave group velocity with the observed flesh pulse speed (see *Supporting Information* for calculations). Using only experimentally measured values of the parameters, we calculate that the F-wave propagates at  $v_{gv} = 1.4 \text{ mm/s}$ . This is in excellent agreement with the observed flesh pulse speed of  $v_{ext} = 1.1 \pm 0.4 \text{ mm/s}$  (mean  $\pm$  SE;  $n = 16$ ). Our model thus properly captures the dynamics of head extension during pulsations.

Regarding the head contraction phase, the main difference to extension is that the head anterior is now in contact with the substrate and thus friction needs to be taken into account. Head contraction dynamics are determined by this competition between muscular relaxation

and friction with the substrate as inertial forces can be neglected. The balance of these two forces defines a relaxation time scale (see *Supporting Information*):

$$\tau_{relax} \cong \eta_{mucus} A_{contact} L_{head}^2 / V_{head} E h_{mucus}. \quad (1)$$

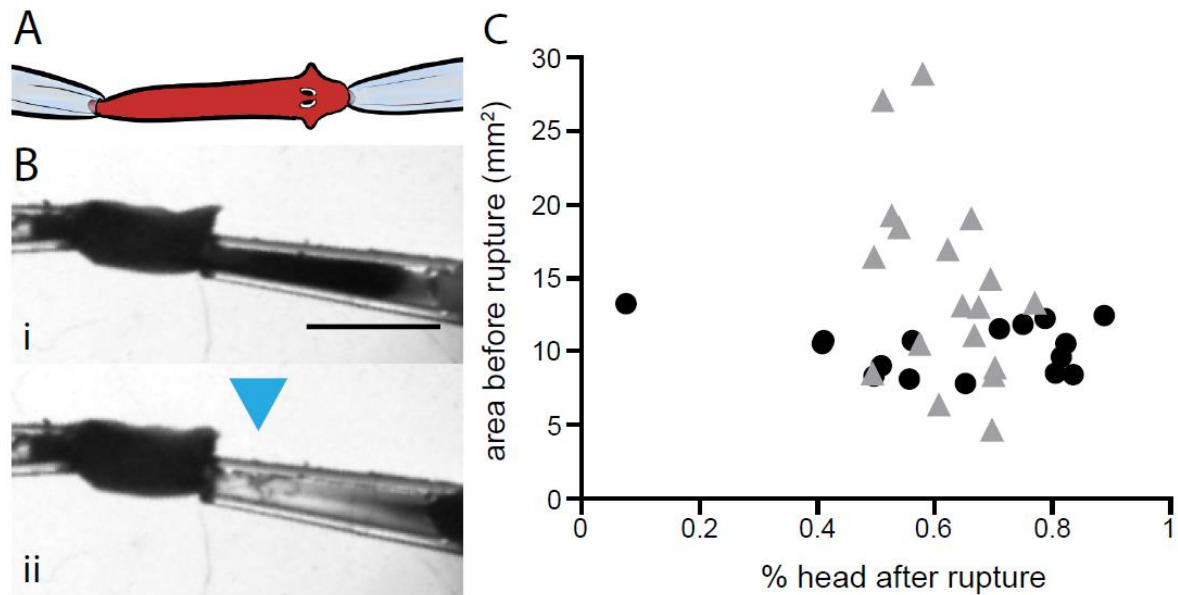
All values in equation (1) were experimentally determined, with  $h_{mucus} = 10 \mu m$  the height of the mucus layer,  $\eta_{mucus} = 65 Pa \cdot s$  the mucus viscosity,  $A_{contact} = 8.7 mm^2$  the surface area of contact with the substrate,  $E = 500 Pa$  the elastic modulus of the shell,  $V_{head} = 22 mm^3$  the head volume and  $L_{head} = 4 mm$  the length of the head (see Table S1). Using these values gives  $\tau_{relax} \cong 82 s$ , which is in reasonable agreement with the experimental value of the relaxation time  $\tau = 44 \pm 20 s$  (*mean*  $\pm$  *SE*;  $n = 18$ ).

Taken together, pulsation timescales are well captured by the linear model, both during elongation and contraction phases, although it is too simple to reproduce the trajectory of pulsations (logistic during elongation, linear during contraction). Head extension is quick, whereas head contraction is slow and thus allows for the build-up of stresses in the waist required for rupture. The model correctly predicts that head extension and waist extension are anti-correlated (Fig. S1), consistent with experimental observations (Fig. 3F, G). During head extension, the waist appears shorter, because it bends vertically (Fig. 3F, G and Movies S4 and S5), whereas during head contraction the waist is extended (Fig. 3F, G) and thus under increased longitudinal stresses. Once these stresses exceed the tissue's yield, rupture occurs. We estimate this critical stress necessary for rupture (see *Supporting Information*) using a linear approach and experimentally determined parameters only (Table S1) and obtain  $\sigma_{waist} \approx 3000 Pa$ , which is in reasonable agreement with our traction force measurements.

**Magnitude of rupture stresses.** The rupture stresses we found are in the kPa range, which is orders of magnitude lower than stresses previously reported for rupture of tissues in other animals (25, 26). It is possible that biological processes proceeding or accompanying waist formation weaken the waist and lower the required rupture stresses. The idea of a pre-defined 'fission zone', with metabolic, cellular or structural differences compared to the rest of the worm, was already suggested over 50 years ago by Child (27) and Tokin (reviewed in (2) and (28)). Hori et al. (3) performed structural analysis of the post-pharyngeal region and observed "presumptive changes in preparation for fissioning" in some, but not all of the samples. Because these samples were fixed planarians without waist, it is impossible to tell if fission would have occurred in the studied region. Thus, experimental evidence demonstrating the existence of a fission zone is absent. Our data on the distribution of the waist location argues against a permanent fission zone, but allows for the possibility that the animal locally prepares for fission before each event., e.g. via enzymatic digestion of extracellular matrix components by metalloproteinases, which have been shown to play a role in planarian tissue homeostasis and regeneration (29, 30).

To test whether such weakening of the fission zone was necessary, we measured the stresses needed to rip a non-dividing planarian apart by applying suction to both ends of a planarian using pipet tips connected to a peristaltic pump (Fig.5A and Methods). In this experiment

(Fig. 5B), a waist was not formed and the time to rupture was much shorter (seconds compared to minutes in fission), rendering biochemically induced structural changes unlikely. However, this pulling experiment yielded comparable stresses as obtained in our traction force measurements, with values ranging between 7.0 kPa and 13.1 kPa. Additionally, we quantified where the worms ruptured and contrasted these measurements with the fission data (Fig.5C). As expected, pulled planarians could tear anywhere along the head-tail axis, including at locations which are “forbidden” zones in fission, such as the very anterior or posterior regions of the animal.



**Fig. 5: Magnitude of rupture stresses. (A) Schematic of the planarian pulling experiment. (B) Representative image of a pulled planarian. (i) right before and (ii) right after rupture. (C) Distributions of rupture planes in fission (black circles, n=22) and pulling (grey triangles, n=16) experiments.**

We also estimated the stresses required to crush a planarian by adding a small weight onto its trunk region (see *Supporting Information*, Fig. S5). Worms were crushed by a 5 gram weight with a contact area of 8.9 mm<sup>2</sup>, thus exerting a stress of  $\sigma=F/A=5.6$  kPa. The results of these both tests indicate that stresses of a few kPa are sufficient to rupture a planarian.

Together, these data show that rupture during fission can be achieved through the planarian’s mechanical properties and the physical mechanisms of pulsations and does not require enzymatic weakening. The planarian’s “trick” of progressive necking is key to successful fission. Once rupture is initiated at these relatively low stresses of a few kPa, the cross-sectional area of the waist decreases further and the internal stresses increase accordingly. We found that the nerve cords break last during fission (Fig.S7). Because their diameter is only 50µm (Fig.S7), this implies stresses in the MPa range, similar to what has been reported as the tensile strength limit for nerve tissues in other organisms (25, 31).

## Discussion and Conclusions

As an active, living biomaterial, *D. japonica* planarians are able to coordinate and execute their own dissection. Our results demonstrate how they can develop sufficiently large tensile stresses to tear themselves apart. Because *D. japonica* are soft and squishy (32) - with an elastic modulus 1000 times smaller than that of nematodes (16, 33) – they are able to tear themselves apart using only substrate adhesion and their own musculature. The self-inflicted rupture is facilitated by the formation of a narrow waist, where tensile stresses are amplified due to reduced cross-sectional area. Friction with the substrate, the mucus rheology and the planarian's elastic modulus are key parameters in determining the dynamics of the contraction phase and, ultimately, rupture. The planarian only needs to exert sufficiently large stresses to break the weakest structures. Once tearing is initiated, the waist cross-sectional area decreases further and tensile stresses are greatly amplified, resulting in stresses able to break stronger anatomical features such as the muscles or nerve cords. From a biomechanical standpoint one could argue that planarians fission because they are soft and thus can do it. However, that's only one part of the story. Planarians fission because they have stem cells which allow them to regenerate the missing structures after the act. Fission is the sole mode of reproduction of the asexual planarians studied here, which poses the question of how this species creates population diversity. Where a planarian divides affects the fitness and reproductive behaviors of its offspring (7, 11–13). Therefore, understanding how fission location is regulated is an important evolutionary question to be answered.

We showed that waist formation/fission location is determined through the relative position of the pharynx. Animals with short RWTs have less time to reposition their pharynx following the previous fission and thus mechanically cannot divide post-pharyngeally, frequently resulting in large tails. What causes some animals to divide rapidly, whereas other comparably sized animals take a long time, remains unknown. However, now that we can predict where an individual will divide, we can start to dissect whether molecular differences exist between pre- and post-pharynx dividers, which could provide insights into the mechanisms controlling RWT and, if existent, a fission trigger.

Finally, going beyond planarian reproduction, this study shows how one can gain insights into complex animal behaviors that are difficult to access experimentally, simply by “watching”. Quantitative image analysis allows for the construction of simple physical models, which can then be tested against the empirical data, potentially revealing new regulatory mechanisms.

## Materials and Methods.

**Planarian Maintenance, Fission Experiments, and Analysis.** Specimens of clonal asexual *Dugesia japonica* were used for all fission experiments. Since planarians do not need a brain to fission, specimen were decapitated to increase the likelihood of fission in the presence of light (34). Details on planarian maintenance, fission experiments and analysis are provided in *SI Materials and Methods*.

**Single Worm Statistical Data.** Fissions were tracked using the SAPling database and barcode system (35) and planarian head and tail sizes were quantified as described in detail in the *SI Materials and Methods*.

**Traction Force Measurements.** Traction forces were measured using custom gel substrates as described in detail in the *SI Materials and Methods*.

**Planarian Pulling.** Planarians were subjected to external stresses using a peristaltic pump (Cole Parmer, Vernon Hill, IL, USA). Upper and lower bounds for the stress applied to the planarian were calculated using the peristaltic pump to lift chrome steel beads. Details are provided in *SI Materials and Methods*.

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## References

1. Hirshfeld AW (2006) *The Electric Life of Michael Faraday* (Walker Books, London). 1st Ed.
2. Brondsted H V. (1955) Planarian Regeneration. *Biol Rev* 30(1):65–126.
3. Hori I, Kishida Y (1998) A fine structural study of regeneration after fission in the planarian *Dugesia japonica*. *Hydrobiologia* 383(1/3):131–136.
4. Morita M, Best JB (1984) Effects of photoperiods and melatonin on planarian asexual reproduction. *J Exp Zool* 231(2):273–282.
5. Paskin TR, Jellies J, Bacher J, Beane WS (2014) Planarian Phototactic Assay Reveals Differential Behavioral Responses Based on Wavelength. *PLoS One* 9(12):e114708.
6. Asano Y, Nakamura S, Ishida S, Azuma K, Shinozawa T (1998) Rhodopsin-like proteins in planarian eye and auricle: detection and functional analysis. *J Exp Biol* 201(9).
7. Carter JA, Lind CH, Truong MP, Collins E-MS (2015) To Each His Own. *J Stat Phys* 161(1):250–272.
8. Nentwig MR (1978) Comparative morphological studies of head development after

- decapitation and after fission in the planarian *Dugesia dorotocephala*. *Trans Am Microsc Soc* 97(3):297–310.
9. Hori I, Kishida Y (2001) Further observation on the early regenerates after fission in the planarian *Dugesia japonica*. *Belgian J Zool (Suppl. 1)*.
  10. Vandel A (1922) *Recherches expérimentales sur les modes de reproduction des planaires triclades paludicoles* (Edition du Bulletin biologique de la France et de la Belgique, Paris). 1st Ed.
  11. Dunkel J, Talbot J, Schötz E-M (2011) Memory and obesity affect the population dynamics of asexual freshwater planarians. *Phys Biol* 8(2):26003.
  12. Quinodoz S, Thomas MA, Dunkel J, Schötz E-M (2011) The More the Merrier? *J Stat Phys* 142(6):1324–1336.
  13. Thomas MA, Quinodoz S, Schötz E-M (2012) Size Matters! *J Stat Phys* 148(4):664–676.
  14. Cebrià F (2016) Planarian Body-Wall Muscle: Regeneration and Function beyond a Simple Skeletal Support. *Front Cell Dev Biol* 4(February):1–10.
  15. Kier WM (2012) The diversity of hydrostatic skeletons. *J Exp Biol* 215(8):1247–1257.
  16. Cochet-Escartin O, Mickolajczyk KJ, Collins E-MS (2015) Scrunching: a novel escape gait in planarians. *Phys Biol* 12(5):56010.
  17. Thompson DW (1992) *On Growth and Form: The Complete Revised Edition* (Dover Publications, Dover). Rev Ed.
  18. Yang X, Kaj KJ, Schwab DJ, Collins E-MS (2017) Coordination of size-control, reproduction and generational memory in freshwater planarians. *Phys Biol* 14(3):36003.
  19. Orii H, Ito H, Watanabe K (2002) Anatomy of the Planarian *Dugesia japonica* I. The Muscular System Revealed by Antisera against Myosin Heavy Chains. *Zoolog Sci* 19(10):1123–1131.
  20. Child CM (1906) The relation between regulation and fission in planaria. *Biol Bull* 11(3):113–123.
  21. Mead RW (1985) Proportioning and regeneration in fissioned and unfissioned individuals of the planarian *Dugesia tigrina*. *J Exp Zool* 235(1):45–54.
  22. Sheĭman IM, Sedel'nikov Z V, Shkutin MF, Kreshchenko ND Asexual reproduction of planarians: metric studies. *Ontogenez* 37(2):130–5.
  23. Glazer AM, Wilkinson AW, Backer CB, Lapan SW, Gutzman JH, Cheeseman IM, Reddien PW (2010) The Zn finger protein Iguana impacts Hedgehog signaling by promoting ciliogenesis. *Dev Biol* 337(1):148–56.
  24. Style RW, Boltyanskiy R, German GK, Hyland C, MacMinn CW, Mertz AF, Wilen LA, Xu Y, Dufresne ER (2014) Traction force microscopy in physics and biology. *Soft Matter* 10(23):4047–55.
  25. Kwan MK, Wall EJ, Massie J, Garfin SR (1992) Strain, stress and stretch of peripheral nerve. Rabbit experiments in vitro and in vivo. *Acta Orthop Scand* 63(3):267–72.
  26. Muiznieks LD, Keeley FW (2013) Molecular assembly and mechanical properties of



- the extracellular matrix: A fibrous protein perspective. *Biochim Biophys Acta - Mol Basis Dis* 1832(7):866–875.
27. Child CM (1911) Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. II. Physiological dominance of anterior over posterior regions in the regulation of *Planaria dorotocephala*. *J Exp Zool* 11(3):187–220.
  28. Krichinskaya EB (1986) Asexual reproduction, regeneration, and somatic embryogenesis in the planarian *Dugesia tigrina* (Turbellaria). *Hydrobiologia* 132(1):195–200.
  29. Isolani ME, et al. (2013) Planarians as a Model to Assess In Vivo the Role of Matrix Metalloproteinase Genes during Homeostasis and Regeneration. *PLoS One* 8(2):e55649.
  30. Sawada T, Oofusa K, Yoshizato K (1999) Characterization of a collagenolytic enzyme released from wounded planarians *Dugesia japonica*. *Wound Repair Regen* 7(6):458–466.
  31. Topp KS, Boyd BS (2006) Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. *Phys Ther* 86(1):92–109.
  32. Dexter JP, Tamme MB, Lind CH, Collins E-MS (2014) On-chip immobilization of planarians for in vivo imaging. *Sci Rep* 4:6388.
  33. Backholm M, Ryu WS, Dalnoki-Veress K (2013) Viscoelastic properties of the nematode *Caenorhabditis elegans*, a self-similar, shear-thinning worm. *Proc Natl Acad Sci U S A* 110(12):4528–33.
  34. Best JB, Abelein M, Kreutzer E, Pigon A (1975) Cephalic mechanism for social control of fissioning in planarians: III. Central nervous system centers of facilitation and inhibition. *J Comp Physiol Psychol* 89(8):923–932.
  35. Thomas MA, Schötz E-M (2011) SAPling: a Scan-Add-Print barcoding database system to label and track asexual organisms. *J Exp Biol* 214(Pt 21):3518–23.
  36. Hagstrom D, Cochet-Escartin O, Zhang S, Khuu C, Collins E-MS (2015) Freshwater planarians as an alternative animal model for neurotoxicology. *Toxicol Sci* 147(1):270–85.
  37. King RS, et al. (2013) In situ hybridization protocol for enhanced detection of gene expression in the planarian *Schmidtea mediterranea*. *BMC Dev Biol* 13(1):8.
  38. Gutierrez E, Groisman A (2011) Measurements of Elastic Moduli of Silicone Gel Substrates with a Microfluidic Device. *PLoS One* 6(9):e25534.
  39. Ross KG, Omuro KC, Taylor MR, Munday RK, Hubert A, King RS, Zayas RM (2015) Novel monoclonal antibodies to study tissue regeneration in planarians. *BMC Dev Biol* 15:2.
  40. Wilson RJ, Skierczynski BA, Meyer JK, Skalak R, Kristan WB (1996) Mapping motor neuron activity to overt behavior in the leech. I. Passive biomechanical properties of the body wall. *J Comp Physiol A* 178(5):637–54.
  41. Junger MC, Feit D (1986) *Sound, structures, and their interaction* (MIT Press,

Cambridge). 2nd Ed.

42. Gilpin W, Uppaluri S, Brangwynne CP (2015) Worms under Pressure: Bulk Mechanical Properties of *C. elegans* Are Independent of the Cuticle. *Biophys J* 108(8):1887–98.