The ejection of internal organs, i.e., evisceration, is a well-known phenomenon in sea-cucumbers. We report the ability of a member of the Chordate phyla, the tropical ascidian *Polycarpa mytiligera*, to eviscerate and regenerate its gut within 12 days, and to rebuild its branchial sac within 19 days. Evisceration occurred within 4–43 seconds of gentle mechanical pressure exerted on the tunic in 47% of the tested *P. mytiligera*. Individuals were able to discard up to 3/4 of their digestive tract via the incurrent siphon by rupture of the branchial sac in this area. Although chemical analysis revealed no significant levels of toxic compounds, the eviscerated guts were unpalatable to the triggerfish and pufferfish on which they were tested, suggesting evisceration as a defense mechanism. Given the close affinity of ascidians to vertebrates, the regeneration pathway of the viscera and branchial sac of ascidians suggests its potential beneficial application in soft tissue regeneration research.

The wide range of regenerative powers within the animal kingdom has drawn the attention of scientists since the early 18th century. From hydras to planarians and geckos, the ability of certain species to redevelop various parts of the body and regain some or all of their original form and function presents fundamental opportunities for research in the fields of cell signaling, development, and adaptation. Holothurians (sea-cucumbers) are well-known for their ability to regularly discard completely or mostly their internal organs in response to an external stimulus, or on a seasonal basis. The enormous progress that has been made in the field of holothurian evisceration research in the past two decades has revealed important molecular mechanisms, cellular pathways, cancer-related gene expression, and organogenesis processes, thus establishing a solid foundation for studies on post-traumatic regeneration. Although the regenerative powers of ascidians, a unique group of filter-feeder marine organisms belonging to the Deuterostomes, have been studied extensively, this has focused mainly on the regeneration of colonial species, and partial body-part regeneration of the siphonal region following artificial amputation. However, in the late 19th century, scientists documented several different species of solitary ascidians lacking their viscera and/or the branchial sac. Sluiter (1885), even named one of the species that lacked both the branchial sac and the gut - *Styeloides abranchiata*. Wiley (1897) commented that in another closely-related species, which he named *Styeloides eviscerans*, the animals survive the evisceration procedure, suggesting that regeneration of the eviscerated body parts (referring mainly to the branchial sac) occurs from the endostyle, a deep ciliated groove along the ventral-mid line of the branchial sac which secretes mucus. Despite the fascinating opportunities to study evisceration in ascidians, this phenomenon was only anecdotally mentioned by taxonomists in the following decades in several other species (see Supplementary Table S1), leaving basic questions unanswered, such as: How are the viscera ejected? Does the animal survive following evisceration? Does it rebuild its viscera; and if so - how? Our documentation of evisceration response of the solitary ascidian *Polycarpa mytiligera* (Savigny, 1816), a conspicuous member on Indo-Pacific coral reefs, a member on Indo-Pacific coral reefs, provides a unique opportunity to deepen our knowledge and revisit the study of evisceration in ascidians, establishing a solid platform from which to study regeneration of the digestive tube from molecular, cellular, and developmental aspects.

**Results**

*Polycarpa mytiligera* is a relatively large species (about 6 cm in length) with a tough brownish tunic covered by epibionts. Following gentle squeezing of the tunic it is able to eviscerate its gut through the incurrent opening (oral siphon, Fig. 1, video S1). We found that this phenomenon occurred in 47% of our attempts to stimulate evisceration (*n* = 66 different individuals), leaving the eviscerated animals in the field in a highly contracted state, with both siphons closed. Marked eviscerated individuals that were followed daily in the field, and individuals that were induced to eviscerate in water tanks, demonstrated the same reaction following evisceration:
phenomenon. The current study is the first to demonstrate this behavior as a natural response to mechanical stress in a solitary tropical ascidian. *Polycarpa mytiligera* is highly abundant on hard surfaces in coral reefs, on which strong-jawed fish such as triggerfish and pufferfish often predate various benthic invertebrates. Thus, it is possible that *P. mytiligera*, which is camouflaged by its epibiont growth, will be accidently bitten, a stimulus resulting in evisceration of the gut in order to either distract the predator fish, or signal the ascidian’s unpalatability. Following evisceration the animal remains in a highly contracted state, which together with its massive epibiont cover provides complete camouflage against an additional attack (video S1). Although ascidians are well known for their ability to accumulate heavy metals, the chemical analysis of the eviscerated gut did not reveal any significant amounts of toxic elements. Yet, our feeding assays revealed an absolute rejection by the tested fish of the eviscerated guts (Table S3), indicating an alternative strategy of *P. mytiligera* by which to induce a repellent response, possibly by secondary metabolites. Predation pressure is a key factor in shaping tropical ecosystem assemblages. Thus, evolving such a defensive trait provides a strong advantage, possibly contributing to the high abundance of *P. mytiligera* in this habitat.

The evisceration response through the incurred siphon leaves the animal with a torn branchial sac (figure 3). The branchial sac is a chamber perforated by dorso-ventral rows of gill slits called stigmata, which are formed during the juvenile stages. Stigma development has been studied in several species since they are considered as key structures for the understanding of the evolution of the deuterostome body plan. Our documentation of the gradual regeneration and recovery of the branchial sac provides researchers with a unique insight into the development of the stigmata in adult individuals, and to the formation of the branchial folds in Stolidobranch ascidians.

The fascinating ability of solitary ascidians such as *P. mytiligera* to completely regenerate their digestive system and repair the branchial sac within a period of less than three weeks, provides a unique insight into the regenerative powers within the Chordate phyla. In view of the growing recognition of the potential of marine organisms to contribute to the treatment of post-traumatic injuries in humans, findings from the study of the evisceration phenomenon in ascidians, which have a close affinity to vertebrates, are expected to contribute to our understanding of the treatment of visceral injuries in humans.

### Discussion

Prior to the observations provided here, evisceration in ascidians has been sporadically noted by ascidian taxonomists as an abnormal phenomenon. The current study is the first to demonstrate this behavior as a natural response to mechanical stress in a solitary tropical ascidian. *Polycarpa mytiligera* is highly abundant on hard surfaces in coral reefs, on which strong-jawed fish such as triggerfish and pufferfish often predate various benthic invertebrates. Thus, it is possible that *P. mytiligera*, which is camouflaged by its epibiont growth, will be accidently bitten, a stimulus resulting in evisceration of the gut in order to either distract the predator fish, or signal the ascidian’s unpalatability. Following evisceration the animal remains in a highly contracted state, which together with its massive epibiont cover provides complete camouflage against an additional attack (video S1). Although ascidians are well known for their ability to accumulate heavy metals, the chemical analysis of the eviscerated gut did not reveal any significant amounts of toxic elements. Yet, our feeding assays revealed an absolute rejection by the tested fish of the eviscerated guts (Table S3), indicating an alternative strategy of *P. mytiligera* by which to induce a repellent response, possibly by secondary metabolites. Predation pressure is a key factor in shaping tropical ecosystem assemblages. Thus, evolving such a defensive trait provides a strong advantage, possibly contributing to the high abundance of *P. mytiligera* in this habitat.

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In order to ascertain how common is the evisceration phenomenon, we attempted inducing evisceration artificially by gently squeezing 66 individuals overgrowing the bottom of a floating dock in Eilat, Red Sea (29°32’51.93”N 34°57’13.47”E) in November 2013. The mechanical pressure was maintained until the animal had either expelled its gut, or for no longer than one minute. Only one attempt was conducted on each individual. To quantify latency to evisceration and to follow the expulsion path of the gut, we photographed and analyzed video-clips of each attempt using a Canon Powershot G15 camera in an Ikelite underwater housing.

Regeneration progress experiment. In November 2013, we marked with tagged plastic wires 12 individuals that had been induced to eviscerate in the field. The discarded gut length of these 12 specimens was estimated by analyzing gut photos using ImageJ image analysis software28. Five gut samples of the largest size group were immediately transferred to −20°C for future ICP-MS chemical analysis. Of the marked individuals in the field, we sampled three individuals weekly and preserved them in 4% formaldehyde solution3. Dissected specimens were studied and photographed using a Nikon SMZ18 stereomicroscope. All specimens are deposited at The Steinhardt Museum of Natural History and National Research Center at Tel-Aviv University (voucher numbers AS25769-AS25810).

Heavy metal analysis. The eviscerated gut samples were digested in a Milestone Ethos 1600 microwave, using 70% HNO₃, 30% HCl and 30% H₂O₂ in Teflon vessels. The analysis was done using Agilent 7700 ICP-MS by Milouda and Migal Laboratories, Israel.

Feeding assays. In order to observe the reaction of coral-reef fish to the eviscerated gut, we ran several feeding trials at the underwater observatory facilities in Eilat. The trials were conducted in February 2014, using five large aquaria that the fish inhabit as part of the regular exhibition. Thus, the fish were well acclimated to this environment and are used to being hand-fed by the staff. We used a number of common strong-jawed fish such as triggerfish and pufferfish (24 individuals total, Table S3) which are used to being hand-fed by the staff. We used a number of common strong-jawed fish such as triggerfish and pufferfish (24 individuals total, Table S3) which are used to being hand-fed by the staff. We used a number of common strong-jawed fish such as triggerfish and pufferfish (24 individuals total, Table S3) which are used to being hand-fed by the staff. 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Author contributions
N.S. and T.G. wrote the main text and conducted the field work. N.S. prepared the figures, and T.G. the supplementary tables. All authors reviewed the manuscript.

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