



## The cutting edge of the lock-and-key hypothesis in weevils: a case study of *Sitona fairmairei* (Coleoptera, Curculionidae)

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**ABSTRACT.** According to a perspective, male and female copulatory organs have not more extreme morphologies. However, a skewed bias in males, -as well as the scanty taxonomic data on female genitalia, may explain why most evolutionary biologists failed to find acceptable evidence for the reproductive co-evolution in insects. A recent finding shows that the mechanical footing has a cooperative essence in the weevil genus *Sitona*. In this viewpoint, successful mating is achieved by the dynamic correspondence between the male endophallus and female genital lumen. The present work addresses some missing evidence in the genital traits of this taxon that may keep the hampered comprehensions under lock and key. The female bursal lumen in the studied species is provisioned with a pair of forklift-shaped structures (named the “codelocks”) that may provide for stabilizing the characteristic position of the male bio-syringe during copulation. This structure varies between the species based on the shape and direction of its paired levers, so that an elaborate algorithm possibly serves as the fuel of this device. However, in one of these, *Sitona fairmairei* (Allard, 1869), the levers perform a dual function in accordance to the lockback mechanism of the male copulatory organ. This species is also featured for having several campaniform mechano-receptors on the flanges of the male bio-syringe which are involved in the in-vivo navigation of this armature towards female spermathecal duct.

**Key words:** Campaniform sensillae, Codelock, Genitalia, Lockback mechanism.

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### INTRODUCTION

Female insects possess membranous and poorly visible internal genitalia that require exhausting samplings. Likewise, these structures have been inadequately studied by taxonomists, perhaps due to a remarkably strong skew mostly reflected a bias for gender-inequality (Ah-King et al., 2014), or the perceived lack of evidence for the co-evolution of male and female genitalia (Eberhard & Ramirez, 2004). Unfortunately, the problem seems to have gotten worse, when the females are assumed to share an invariant genitalia, while so many neutrally evolved (pleiotropic) keys have continued to flourish (Brennan & Prum, 2015). It's hardly surprising that the literatures dealing with insects genitalia pay far too little attention to the role of females in sexual dynamics (Méndez & Córdoba-Aguilar, 2004; Mikkola, 2008; Rudoy et al., 2016). However, several hypotheses have been put forward to explain these enigmas and the conflicts have greatly expanded since then. Hosken & Stockley (2004) believed that the concept of sexual selection is the main driving force behind the evolution of genitalia. Shapiro & Porter

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(1989), in an influential review, explained why the configuration of the lock-and-key hypothesis is frequently dismissed as unimportant in genital evolution due to the apparent lack of variation in female genitalia across the taxa and some pleiotropic effects of selection acting on general morphology. However, they expressed their interest in future observations on testing the given hypothesis based on the genital neuroanatomy. Some studies also showed that few variations of male genitalia may arise as the negative pleiotropic side effects of adaptations that yield other selective advantages to males during the reproductive competition (Morrow et al., 2003). Zhou et al. (2020) concluded that, genital diversity can bridge the gap between the morphological and the biological species concepts, regarding the recently reactivated lock-and-key hypothesis. They also outlined various processes that are considered by evolutionary biologists as the routes of adaptive changes towards complexity. Alichì (2021) and Alichì & Minaei (2021) brought together some experimental findings on *Sitona* Germar, 1817, and distinguished the novel concept of the “cooperative lock-and-key” in this taxon. They postulated that the male and female copulatory organs are not mechanically exclusive, and highlighted the cooperative match in *Sitona fairmairei*. In the present work, the author has tried to step on this challenging road once again, and put forward new findings in the genital matching of the studied *Sitona* species, involving lost evidence for co-evolution in this taxon. Among them, the amazing “codelocks” are considered as the cutting edge of the lock-and-key hypothesis, whose functional roles are discussed here for the first time in insects. In all probability, such device constitutes a joint character among the coleopterans having developed transfer apparatuses and direct sperm transference. In this way, the campaniform mechano-receptors on the male bio-syringe are also supposed to be present in this group of organisms.

## MATERIAL AND METHODS

Collected weevils were kept for two hours in Bouins solution to promote the preservation of the internal organs. They were then transferred to 70% ethyl alcohol and were allowed to stand for a waiting period before dissection. The whole abdomen was removed from the sample and boiled for five minutes in 10% potassium hydroxide (KOH) to provide a better view of the internal structures. After washing in distilled water, the sample was placed in acetocarmine for several minutes to stain the soft tissues. The formerly discussed protocol (Alichì, 2021) was followed for preparing mounts from the copulatory organs. In this case, the dissected parts of the male or female genitalia were washed in distilled water and dehydrated through ethyl alcohol series, and then transferred to clove oil followed by xylol for clearing. Finally, they were transferred to the slides and mounted in canada balsam. The photographs were made using an Olympus BX51 phase-contrast microscope with a DP27 digital camera and cellSens software.

## RESULTS

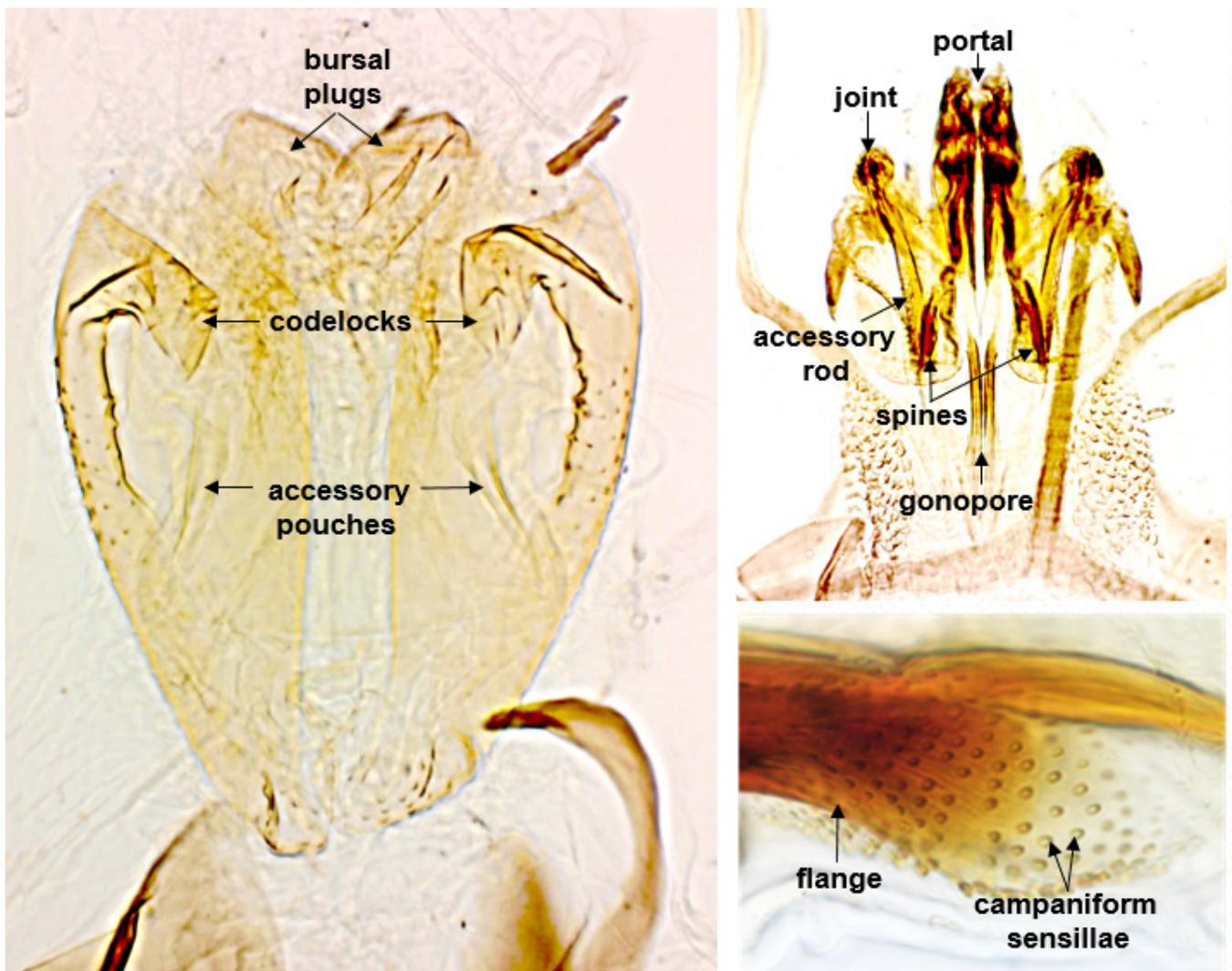
### *In-vivo navigation by the male transfer apparatus*

The so called “bio-syringe” of *Sitona fairmairei* is approximately 200 microns long and made up of 15 subunits (sclerites) which have been shaped into three functional zones that altogether form an integrated transfer apparatus (Fig. 3). Recently, a cooperative match between the ejaculatory pump of the male transfer apparatus (Fig. 3) and the female “bursal plugs” (Fig. 1) in this species was explained. However, a lockback structure and the group of mechano-receptors found on the lateral processes (flanges) of the male bio-syringe in this species (Fig. 1) are described herein for the first time. In repose condition, a pair of “accessory rods” are articulated at the dorsal tips of the portal branches and are held at the sides of the endophallic armature by a lockback design which is essentially comprised of a paired “spines” originated from the sclerotized elements of the flanges (Figs. 1, 3). However, during coitus, the protracting muscles attached to the male copulatory system induce a 180° inversion in the frontal plane of the internal sac and its armature, so that the accessory rods move to the ventral side, in the opposite direction of the pump assembly (Fig. 3). A group of mechano-receptors (campaniform

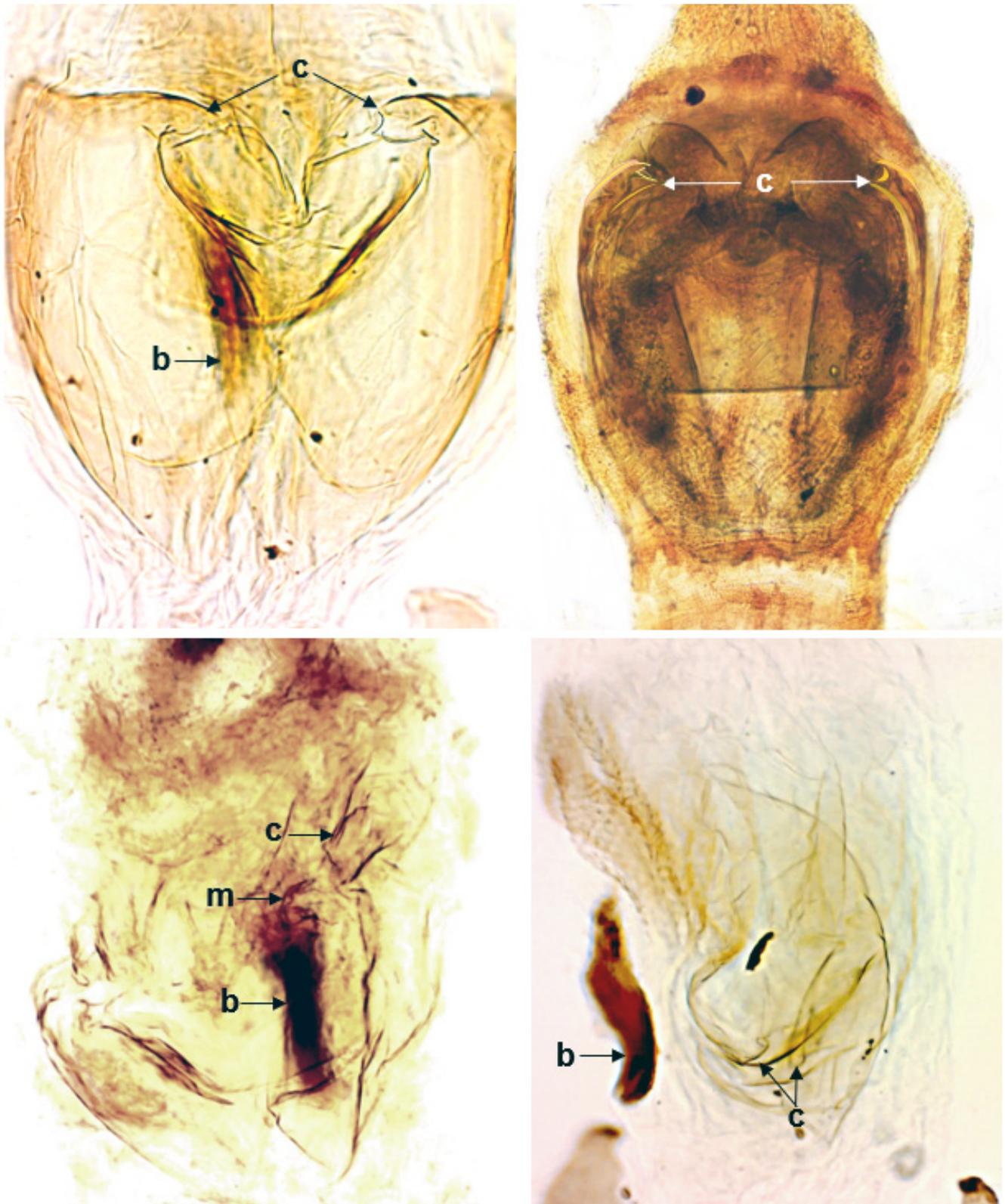
sensillae) was observed on the club-like extension of each flange, as well, which control the posture of the transfer apparatus during its navigation inside the female genital lumen (Fig. 1). They respond best to compression and the neighboring sensillae may have different sensitivities to strain depending on their orientation in the cuticle.

### *Evolution of the codelocks in the female bursal lumen*

A pair of “accessory pouches” in the female genital chamber of *Sitona fairmairei* provide the actual positions for the anchoring device (accessory rods) of the male transfer apparatus (Fig. 1). Anterior to the accessory pouches, there are two forklift-shaped ingrowths (named the “codelocks”) suspended from the lateral walls of the female bursal lumen in this species (Figs. 1, 3). Each codelock is made up of two levers connected at one fixed end and oriented at different directions in various studied species of this genus (Fig. 2). A muscle bundle is originated from the fixed end of each codelock, and is attached to the “baculus” sclerite at the ventral wall of the female genital lumen (Fig. 2). The mechanism behind the codelocks in *S. fairmairei* is that when the male bio-syringe enters the bursal lumen, the muscles attached to the fixed end of the device are stretched and trigger an action potential for muscle contraction, that results in pulling levers down.

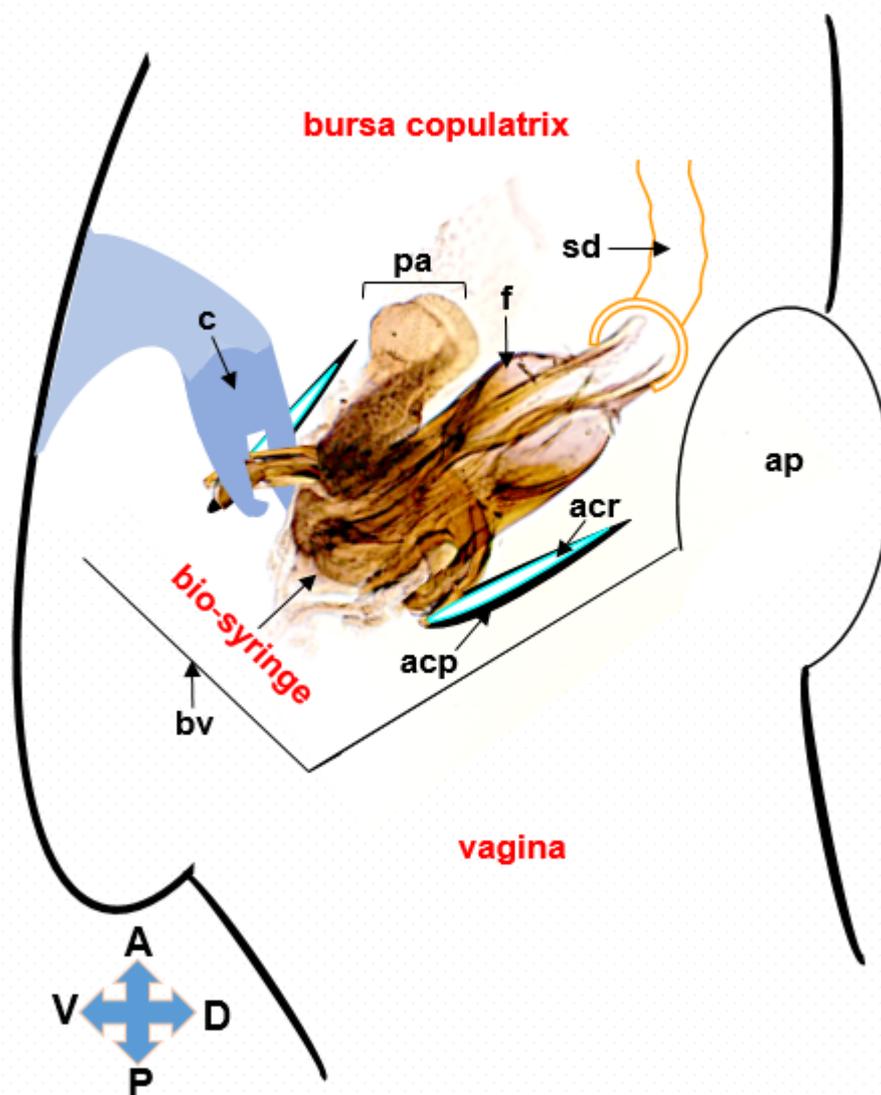


**Figure 1.** Ventral view of the female genital chamber (left), dorsal view of the reposing male transfer apparatus (top-right), and the closer view of a flange (bottom-right) in *Sitona fairmairei* (Allard) (Coleoptera: Curculionidae).



**Figure 2.** Female genital chambers in *Sitona cylindricollis* Fahraeus (top-left, dorsal view), *Sitona tenuis* Rosenhauer (bottom-left, ventral view), *Sitona puncticollis* Stephens (top-right, dorsal view) and *Sitona callosus* Gyllenhal (bottom-right, lateral view) (Coleoptera: Curculionidae), showing variations of the codelocks. **c**: codelock, **b**: baculus, **m**: muscle bundle.

The codelock levers in the majority of the studied *Sitona* species (such as *S. cylindricollis* Fahraeus, *S. tenuis* Rosenhauer, *S. puncticollis* Stephens and *S. callosus* Gyllenhal) (Fig. 2) are designated for grasping the lateral processes (flanges) of the male transfer apparatus and stabilize the bio-syringe during copulation. However, in *S. fairmairei* the levers are unequal (Fig. 1) and perform a dual function. This species is featured for having fused flanges on its bio-syringe, therefore, the flanges can not be secured by the codelock levers. Hence, the levers of each codelock shift to grab the corresponding lateral branch of the male bio-syringe at the point located near the joint (Figs. 1, 3). In this way, the longer lever pushes-off the trapped accessory rod, so that a side flexion of this blade is resulted and the accessory rod can be detached from the aligned spine (lockback structure) and penetrate into the corresponding accessory pouch embedded in the anterior wall of the female bursal valve (Figs. 1, 3).



**Figure 3.** Diagrammatic lateral view of the female genital chamber and the characteristic position of the male bio-syringe in the bursal lumen of *Sitona fairmairei* (Allard) (Coleoptera: Curculionidae). The bursal plugs and one of the codelocks are not shown. **acp**: accessory pouch, **acr**: accessory rod, **ap**: anterior pouch, **bv**: bursal valve, **c**: codelock, **f**: flange, **pa**: pump assembly, **sd**: spermathecal duct. The indicator below shows directions of **A**: anterior, **D**: dorsal, **P**: posterior and **V**: ventral.

## DISCUSSION

The lock-and-key hypothesis would predict that changes in female genital anatomy should be closely tracked by changes in male genital morphology (Mikkola, 2008). However, a penalty notice should be issued when the whole intromittent organ of the male insect is considered to act as a key. It's just a reminder that this structure is comprised of two parts: a membranous internal sac and the supplementary armature which bears the functional orifice of the sac and together with it form the endophallus (Sharp & Muir, 1912; Tuxen, 1970). On the front line of the male intromittent organ lies the intrinsic key, the transfer apparatus, so that the internal sac and all other parts of the copulatory system are merely accessory to it. The functional role of the internal sac is comparable to the human hand that holds the key and tries to secure it during unlocking operation. Upon entering into the female genital chamber, the three-dimensional and elastic internal sac displays a phenotypic plasticity that can change dynamically during interactions with female genital tissues (Kelly & Moore, 2016). Meanwhile, according to the "induced fit" activity in the enzyme receptors (Koshland, 1994), the female genital tract can change its conformation upon confronting the male intromittent organ, like "a glove that changes its shape when a hand slips into it". Hence, the lock-and-key mechanisms in a living system are not solidly based, as it is expected from the mechanical locks and keys. In this regard, the genus *Sitona* is one of the extraordinary exceptions, which has achieved a perfect match between the male and female copulatory organs. In *S. fairmairei*, the female codelocks interact both with the lockback design of the male bio-syringe, as well as having their role in stabilizing the characteristic position of this armature throughout mating (Fig. 3). It seems likely that the campaniform organs observed in this species, allow the lateral processes (flanges) of the male bio-syringe to function as a gyroscope, analogous to the same sensillae on the base of the halteres in Diptera (Agrawal et al., 2017). Sensory feedback from these organs is integrated in the control of posture and the dome shape of the sensillae makes them directionally selective (Zill et al., 2013). As a result, the aspect of a cooperative match in this group of organisms would be significantly to the advantage of the female through a key decision for acceptance or rejection of the potential mate, and controlling the steady state of gamete transfer to insure successful fertilization. However, much can be learned by future studies on the male and female genital tracts of Coleoptera over their entire range.

## AUTHOR'S CONTRIBUTION

M.A. designed and performed all practical aspects of the work and writing the manuscript. He read and approved the final version of the manuscript.

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## AVAILABILITY OF DATA AND MATERIAL

Not applicable.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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## لبه تیز نظریه قفل و کلید در سرخرطومی‌ها: مطالعه‌ای بر روی *Sitona fairmairei* (Coleoptera, Curculionidae)

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**چکیده:** بر اساس یک دیدگاه، اندام جفت‌گیری در حشرات نر و ماده ریخت شناسی کاملاً متفاوتی ندارند. هرچند تعصب موجود در مورد جنس نر و نیز کمبود اطلاعات تاکسونومیکی در رابطه با جنس ماده، می‌تواند توضیح مناسبی مبنی بر اینکه چرا اکثر زیست‌شناسان تکاملی نتوانسته‌اند مدارک قابل قبولی از هم‌تکاملی تولید مثلی در حشرات را بدست آورند باشد. یک یافته جدید نشان می‌دهد که جفت شدن اندام‌های تناسلی در سرخرطومی *Sitona* بر مبنای همیاری دو جنس و تطابق دینامیک بین اندوفالوس نر و مجرای تناسلی ماده می‌باشد. در مطالعه حاضر به چندین گواه مفقوده از خصوصیات تولید مثلی در این گروه اشاره شده که می‌توانند به استنباط‌های متضاد پایان دهند. مجرای جفت‌گیری در حشرات ماده مورد مطالعه در جنس *Sitona* مجهز به یک جفت اندام چنگک مانند (به نام رمز-قفل) بوده که کاربرد آن در تعادل جنیتالیایی نر هنگام جفت‌گیری است. این ساختار در بین گونه‌های مختلف براساس شکل و جهت دو اهرم متصل به آن متفاوت و احتمالاً از یک الگوریتم دقیق پیروی می‌کند. هرچند در گونه *S. fairmairei* این اهرم‌ها به دلیل وجود یک ساز و کار ضامن در جنیتالیایی دارای کاربردی دوگانه می‌باشند. از دیگر خصوصیات ویژه در این گونه، وجود تعدادی گیرنده مکانیکی گنبدی شکل روی لبه‌های جانبی بیو سرنگ حشره نر بوده که در عمل پیمایش درونی آن به سمت مجرای کیسه ذخیره اسپرم ماده دخالت دارند.

**واژگان کلیدی:** گیرنده مکانیکی گنبدی شکل، رمز-قفل، جنیتالیا، ساز و کار ضامن.