

## Genitalic Evolution in Opiliones

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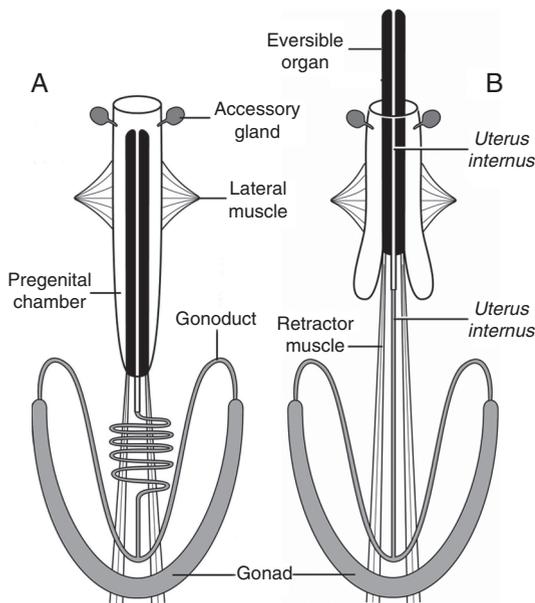
### MORPHOLOGY AND SYSTEMATICS OF OPILIONES

The Opiliones, usually known in English as harvestmen or daddy longlegs, are the third largest group in the class Arachnida, with nearly 6,000 described species (Machado et al. 2007). Harvestmen are a common and nearly ubiquitous component of terrestrial environments, being found in all continents, except Antarctica, from the equator to subpolar regions. They occur in a great variety of habitats in most terrestrial ecosystems, including soil, moss, leaf litter, under rocks, stones, and debris, on vertical surfaces from tree trunks to stone walls, among grassy clumps, and high vegetation. Although some species are widely distributed and can be found in a wide range of habitats, many are much more limited in geographic distribution and habitat use, especially in tropical areas. Some species are restricted to caves, and others occur in very specific microhabitats, such as nests of leaf-cutter ants, (see Curtis & Machado 2007).

The harvestman body is compact and has two main parts, an anterior prosoma (or cephalothorax) and a posterior opisthosoma (or abdomen), with a broad and sometimes poorly defined juncture. This body plan contrasts with that of spiders, whip spiders, whip scorpions, and certain other arachnids in which the prosoma and

opisthosoma are separated by a distinct constriction. The prosoma bears the chelicerae, pedipalps, and four pairs of legs, with the second typically elongated and used as a sensory appendage (Shultz & Pinto-da-Rocha 2007). The dorsal plate of the prosoma, the carapace, generally has a pair of median eyes, but visual acuity is likely poor in most groups (see Acosta & Machado 2007). A pair of defensive glands opens laterally on the carapace, a feature unique to the order (Gnaspini & Hara 2007). The genital opening is located ventrally on the second opisthosomal somite, which has shifted anteriorly relative to the dorsal parts, and lies between the last pair of legs (Shultz & Pinto-da-Rocha 2007).

The basic architecture of the male and female reproductive tract has been described by Shultz & Pinto-da-Rocha (2007). The testis and ovary are both U-shaped with a mesodermal gonoduct (sperm duct, oviduct) emerging from each side (figure 13.1). These gonoducts fuse and continue anteriorly as a single duct, which eventually merges with a cuticle-lined tube that travels through either an eversible penis or ovipositor (figure 13.1). The posterior end of the penis and ovipositor attach to the posterior end of a pregenital chamber formed by flexible cuticular walls that often bear accessory glands. The walls of the pregenital chamber have muscles that seem to expand the pregenital lumen and initiate



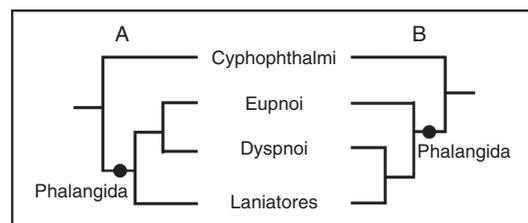
**FIGURE 13.1** Schematic representation of the reproductive system of both male and female harvestmen in ventral view showing the eversible organ (A) inverted and (B) everted. Gonad = testis (♂) and ovary (♀); gonoduct = sperm duct (♂) and oviduct (♀); *uterus internus* = *vas deferens* (♂) and *uterus* (♀); *uterus externus* = propulsive organ + ejaculatory duct (♂) and propulsive organ + vagina (♀); eversible organ = spermatopositor or penis (♂) and ovipositor (♀). Based on de Graaf (1882) and Kästner (1935).

eversion (figure 13.1), which is most likely completed by pressure of the hemolymph. Retraction, on the other hand, is accomplished by a pair of muscles that originate at a posterior tergite and attach to the proximal end of the eversible organ (figure 13.1).

Recent phylogenetic studies place Opiliones near the orders Scorpiones, Pseudoscorpiones, and Solifugae, forming a clade called Dromopoda united by synapomorphies associated with the appendages and mouthparts (Shultz 1990, 2007). Currently, there are two hypotheses for the relationship among the suborders of Opiliones (figure 13.2). One proposes that the suborders Eupnoi and Dyspnoi form a monophyletic group called Palpatores (Shultz 1998; Shultz & Regier 2001). The other unites the suborders Dyspnoi and Laniatores in a monophyletic group

called Dyspnolaniatores (Giribet et al. 1999, 2002, but see Giribet et al. in press). Regardless of the system of higher classification, we recognize 45 families and about 1,500 genera of Opiliones (Giribet & Kury 2007). This diversity is not evenly distributed among the suborders, with Eupnoi and Laniatores comprising together nearly 90% of the species in the order.

Representatives of the suborder Cyphophthalmi are generally small (1 to 3 mm in body length), short-legged, heavily sclerotized inhabitants of soil and caves (Giribet 2007). The suborder Eupnoi comprises two superfamilies: the Phalangioidea, which includes the long-legged forms widely known in the Northern Hemisphere, and the Caddoidea, a small group easily recognized by their huge eyes and spiny pedipalps (Cokendolpher et al. 2007). The suborder Dyspnoi also comprises two superfamilies, Ischyropsalidoidea and Trogluloidea, which are mainly distributed in the Northern Hemisphere (Gruber 2007). The suborder Laniatores is a diverse lineage of armored harvestmen, typically with large and spiny pedipalps (Kury 2007). This suborder is divided into two infraorders: Insidiatores, comprising the superfamilies Travunioidea and Triaenonychoidea, and Grassatores, encompassing the superfamilies Zalmoxoidea, Biantoidea, Phalangodoidea, Epedanoidea, and Gonyleptoidea (Giribet & Kury 2007). However, because our goal is to focus on genitalic diversity across suborders, we will tend to avoid details on the relationships of the superfamilies.



**FIGURE 13.2** The two current hypotheses for the relationship among the suborders of Opiliones. One of them (A) proposes that the suborders Eupnoi + Dyspnoi form a monophyletic group called Palpatores (Shultz 1998; Shultz & Regier 2001), whereas the other (B) proposes that Dyspnoi + Laniatores form a monophyletic group called Dyspnolaniatores (Giribet et al. 1999, 2002).

## MORPHOLOGY AND FUNCTION OF GENITALIA IN OPILIONES: A MACRO-EVOLUTIONARY PLAY IN FOUR ACTS

### Reproductive Strategies: Where We Lay Our Scene

Our knowledge of reproductive strategies in harvestmen has recently been reviewed by Machado & Macías-Ordóñez (2007), based on information available for two species of *Cyphophthalmi*, five of *Eupnoi*, four of *Dyspnoi*, and nine of *Laniatores*. A few more recent studies have also been considered in this chapter (Willemart et al. 2006; Buzatto & Machado 2008; Nazareth & Machado 2009). In this section, we summarize the reproductive strategies recorded for each suborder, which will provide the context for addressing the evolution of genitalia in Opiliones.

The great majority of harvestmen reproduce sexually, although some species reproduce asexually by parthenogenesis (e.g., Phillipson 1959; Tsurusaki 1986). All sexually reproducing species studied so far are polygynandrous, i.e., both males and females engage in copulation with multiple mates frequently within a single day, throughout their reproductive lives (e.g., Edgar 1971; Mora 1990; Macías-Ordóñez 1997; Buzatto & Machado 2008). Fertilization is internal, and mature spermatozoa are immobile (Morrow 2004). Courtship before intromission is often quick and tactile, but in some cases males may offer a glandular nuptial gift before copulation (Martens 1969). Many studies also mention intense courtship during intromission and mate guarding after copulation (reviewed in Machado & Macías-Ordóñez 2007; see also Nazareth & Machado 2009). Additionally, males of many species defend territories, which are used by females as oviposition sites (Mora 1990; Machado & Oliveira 1998; Macías-Ordóñez 1997; Buzatto & Machado 2008). Given the complexity of the male genitalia and the enormous diversity of sexual dimorphism in the order, sexual selection (be it intra- or intersexual) has most likely played a major role in the evolution of harvestmen, as has been proposed for many other groups (e.g., Hosken & Stockley 2004; Cueva del Castillo & Núñez-Farfán 2008).

Males have been shown to produce spermatophores in at least two genera of *Cyphophthalmi*, *Cyphophthalmus* and *Stylocellus* (Karaman 2005;

Novak 2005; Schwendinger & Giribet 2005), and this may be the rule throughout the suborder. Roughly half the gametes are allocated to protective layers around the viable sperm, and covered by a mucous secretion from accessory glands of the reproductive tract (Juberthie & Manier 1978). When compared with other arachnids (reviewed in Proctor 1998), spermatophore production in *Cyphophthalmi* seems to represent a relatively large investment (around 3% of the body volume in *Cyphophthalmus*, based on Fig. 1 from Karaman 2005).

All records on mating in the suborder *Eupnoi* include copulation by means of fully intromittent male genitalia (reviewed in Machado & Macías-Ordóñez 2007). Copula duration is highly variable and in some cases consists of repeated genital intromissions while the male keeps his long, sexually dimorphic pedipalps clasped to the base of female legs II (occasionally I, III or IV), near the trochanter (e.g., Bishop 1949, 1950; Edgar 1971; Macías-Ordóñez 1997). This form of male grasping has been described for three species of *Leiobunum* (see Machado & Macías-Ordóñez 2007), but it seems to be ubiquitous in *Eupnoi* (but see Willemart et al. 2006). When copulation occurs, female cooperation seems to be evident in many cases since females are able to reject intromissions by lowering the prosoma against the substrate thus obstructing the entrance to their genital opening (Immel 1955; Edgar 1971; Macías-Ordóñez 1997). Interactions between male genitalia or chelicerae and the female's mouthparts, as well as grasping of the male genitalia by the female, suggest that some kind of nuptial gift is also obtained by the female (Willemart et al. 2006; see also below).

Only five descriptions of copulation in the suborder *Dyspnoi* exist, but most of them are fairly detailed and show similarities and differences with *Eupnoi*. In at least one species of *Paranemastoma* (Nemastomatidae, Troguloidea) and two of *Ischyropsalis* (Ischyropsalididae, Ischyropsalidoidea) the bases of the male chelicerae are either offered or somewhat forced into the female's mouth, after which the female obtains a secretion from cheliceral glands (Martens 1969; Meijer 1972). Precopulatory interactions seem to be intense in some species, including male tapping on the female's back, and copulation occurs in a face-to-face position, much as in *Eupnoi* (Immel 1954; Martens 1969). On the other hand, full intromission in Troglidae occurs in a belly-to-belly position, and

females may be able to reject the male by lowering the anterior end of the body (Pabst 1953). Dyspnoi males seem to rely less on powerful grasping to negotiate with the females and more on precopulatory courtship, including nuptial gifts. Copulatory courtship such as that reported in Eupnoi has been described in all species of Dyspnoi that have been studied, raising the possibility that females may also exert cryptic choice (Pabst 1953; Immel 1954; Martens 1969). The amount and quality of the secretion offered may be the subject of female evaluation and may influence paternity (Machado & Macías-Ordóñez 2007).

In contrast to the Dyspnoi, courtship before intromission is generally quick and tactile in the Laniatores. Courtship during intromission, on the other hand, involve leg tapping and rubbing and may be intense (reviewed in Machado & Macías-Ordóñez 2007; see also Nazareth & Machado 2009). Like the Eupnoi, copulation occurs in a face-to-face position, but in Laniatores males grasp the females' pedipalps, and not their legs. So far, there is no evidence that the male offers any glandular secretion as a nuptial gift (e.g., Miyosi 1941; Matthiesen 1983; Mora 1990; Buzatto & Machado 2008; Willemart et al. 2008; Nazareth & Machado 2009). Oviposition generally occurs immediately after copulation, with the male remaining close to the female, waving his second pair of legs over her and occasionally tapping her legs and dorsum. Mate guarding may last more than 24 hours, during which the male often tries to copulate, and occasionally succeeds. This pattern is especially common in species with maternal care belonging to the subfamily Goniosomatinae (Gonyleptidae), in which a single male can monopolize a harem containing up to six egg-guarding females (e.g., Machado & Oliveira 1998; Buzatto & Machado 2008). In species with paternal care, such as *Zygopachylus albomarginis* (Manaosbiidae), females court egg-guarding males, which sometimes reject females without copulating. As might be expected, *Z. albomarginis* males display behavioral paternity-assurance strategies, including repeated copulations, postcopulatory female guarding, and coercion to lay eggs soon after copulation (Mora 1990).

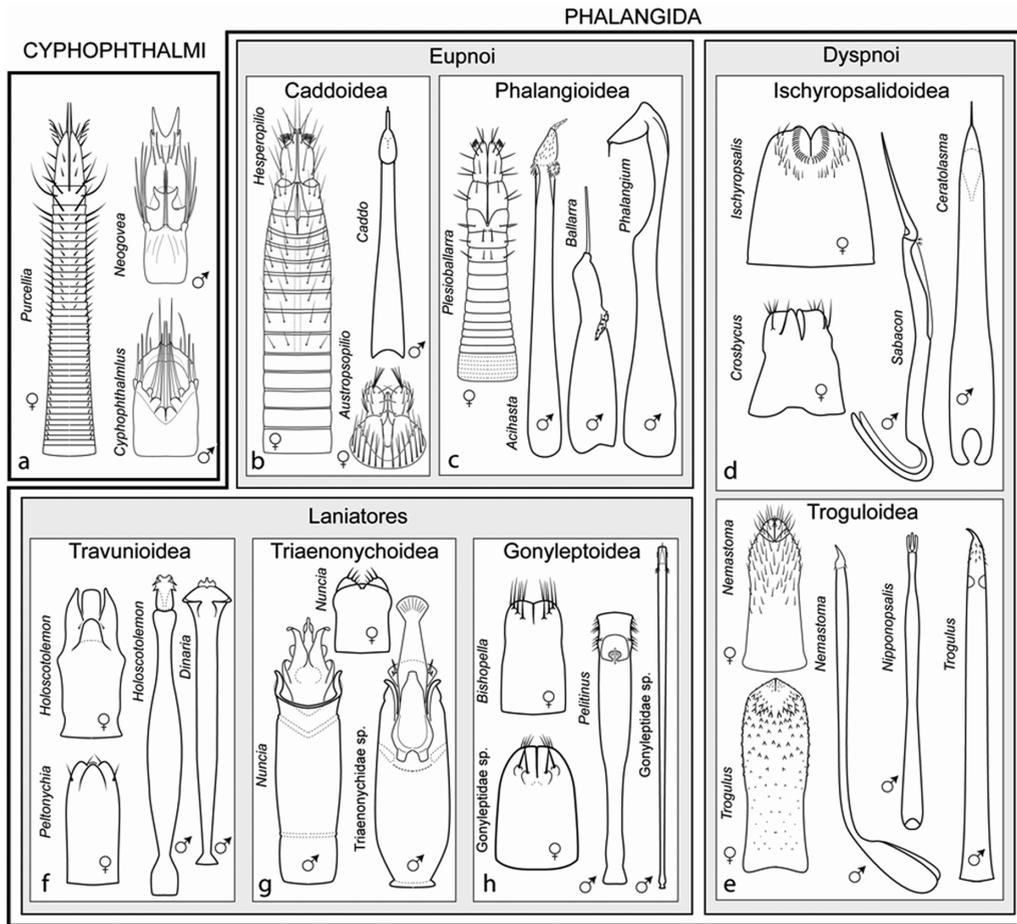
#### The (sexual) Characters: An Ovipositor and a Penis

Most of the research on the reproductive morphology of Opiliones has focused on the structure of

the penis and, to a lesser extent, the ovipositor, and inspired primarily by the search for characters of taxonomic or phylogenetic significance. The eversible genitalia of male Cyphophthalmi is often called a penis, but is much shorter than that of other harvestmen (figure 13.3a) and appears to be used in the transfer of spermatophores rather than direct copulation (see below). Consequently, we follow Van der Hammen (1985) in calling this structure a spermatopositor. Unlike the other groups, the spermatopositor has an extensive array of internal muscles and a ring of projections resembling setae (Martens 1986).

The penis of the non-Cyphophthalmi harvestmen, the Phalangida, is typically divided into two main parts: *pars basalis* and *pars distalis*. The *pars basalis* corresponds to most of the long shaft called *truncus*; the *pars distalis* contains the distal end of the *truncus* and the terminal or subterminal glans (figure 13.4). The *pars distalis* is the part that interacts with the ovipositor and is often equipped with spines, sensilla, and other projections, some highly asymmetrical. The glans is the most variable structure of the penis and contains the opening of the *ductus ejaculatorius*, located at the end of the *stylus* (figure 13.4). Typically in Eupnoi and Dyspnoi, the *pars distalis* is composed almost exclusively of a relatively simple glans with an apical *stylus*, with the glans being only slightly differentiated from the *truncus* (figures 13.3B–E; 13.4). The plesiomorphic glans in Laniatores (e.g., Travunioidea) is a little more complex and differentiated from the *truncus* (figure 13.3F). In the remaining Laniatores, the glans is much more complex, with sclerites that vary widely among the families (figures 13.3G,H, 13.4). The sclerites associated with the distal end of the *ductus ejaculatorius* are called *capsula interna* (figures 13.4B–H). Generally the *capsula interna* is surrounded totally or partially by the *capsula externa* formed simply by a soft sac called *follis* (figures 13.4F–H) or by highly modified structures such as the titillators (figure 13.4E) and the *stragulum* (see details in Act IV).

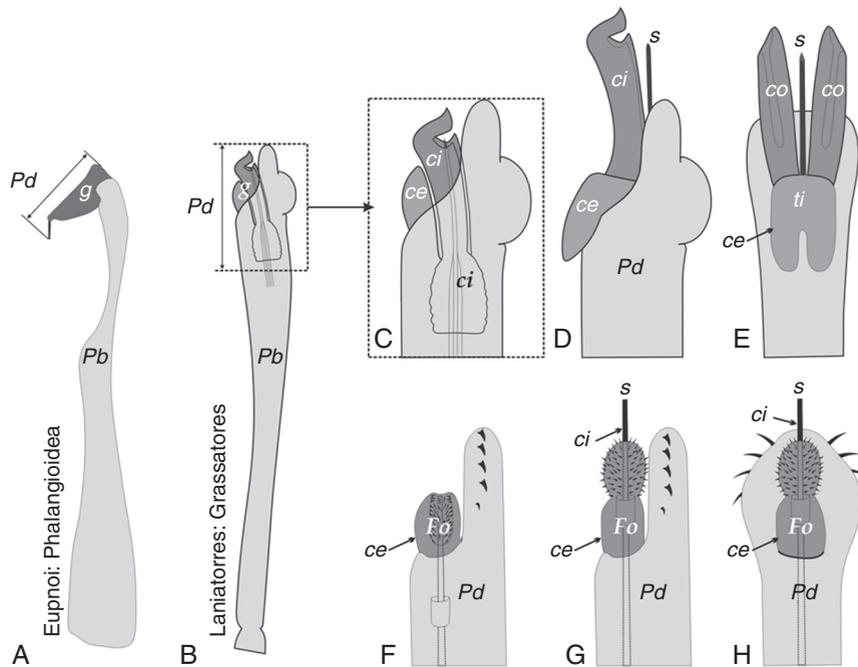
In muscular penes, the movement of the glans relative to the *truncus* is provided by one or two intrinsic muscles that originate from the shaft and insert on a cuticular tendon that ends at the base of the glans. This muscular type of penis occurs in the Eupnoi and Dyspnoi, as well as in the superfamilies Travunioidea and Triaenonychoidea within Laniatores (figures 13.3b–g). Grassatores, on the other hand, have a hydraulic penis, i.e., the intrinsic



**FIGURE 13.3** Male and female genitalia from representatives of the order Opiliones. (a) Cyphophthalmi: *Purcellia*, ovipositor (Hansen & Soerensen 1904); *Neogovea*, *Cyphophthalmus*, spermatopositors (Martens 1986). (b) Caddoidea: *Hesperopilio*, ovipositor (Shultz & Cekalovic 2006); *Austropsopilio*, ovipositor (Shultz & Cekalovic 2003); *Caddo*, penis (Gruber 1975). (c) Phalangioida: *Pseudoballarra*, ovipositor; *Acihasta*, *Ballarra*, penes (Hunt & Cokendolpher 1991); *Phalangium*, penis (de Graaf 1882). (d) Ischyropsalidoidea: *Ischyropsalis*, ovipositor (Hansen & Soerensen 1904); *Crosbyicus*, ovipositor (Shear 1986); *Sabacon*, penis (Martens 1986); *Ceratolasma*, penis (Gruber 1978). (e) Troguloidea: *Nemastoma*, ovipositor (Hansen & Soerensen 1904); *Trogulus*, ovipositor (Martens et al. 1981); *Nemastoma*, *Trogulus*, penes (Hansen & Soerensen 1904), *Nipponopsalis*, penis (Martens & Suzuki 1966). (f) Travunioidea: *Peltonychia*, *Holoscotolemon*, ovipositors (Martens 1986); *Holoscotolemon*, *Dinaria*, penes (Martens 1976). (g) Triaenonychidae: *Nuncia*, ovipositor, penis (Muñoz Cuevas 1972); *Triaenonychidae* sp., penis (Martens 1976). (h) Gonyleptoidea (representative of the infraorder Grassatores): *Gonyleptidae* sp., *Bishopella*, ovipositors (Martens et al. 1981); *Pelitinus*, *Gonyleptidae* sp., penes (Martens 1976). All figures redrawn.

muscles are absent and the glans is apparently operated by internal hemolymph pressure (e.g., Gonyleptoidea; figure 13.3h). It is important to note, however, that the terms “muscular” and “hydraulic” refer to the operation of the glans only;

eversion and inversion of the entire penis in all harvestmen are apparently achieved by a combination of muscular and hydraulic mechanisms (Martens 1986; Shultz & Pinto-da-Rocha 2007). Seminal products are apparently pushed through the long



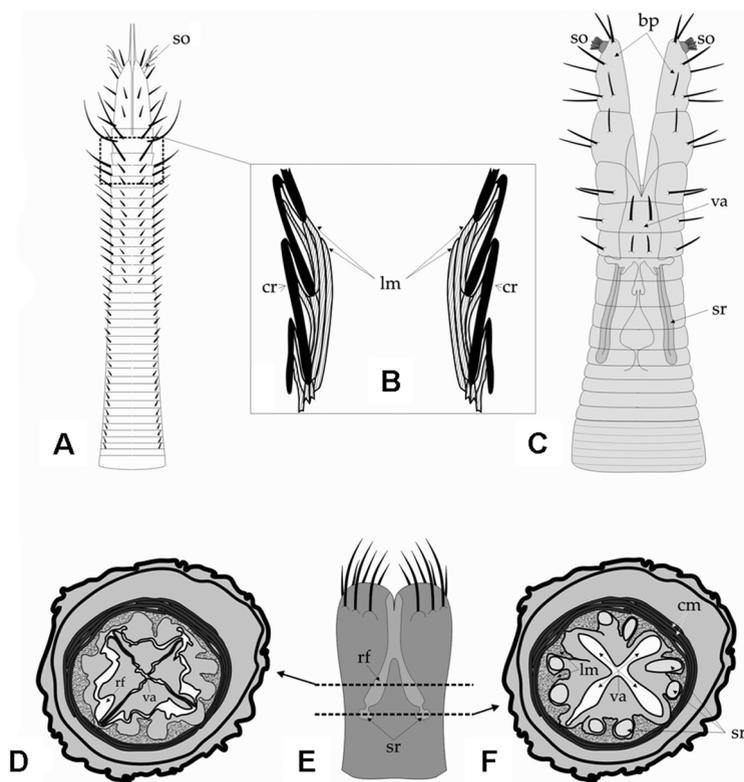
**FIGURE 13.4** Schemes contrasting the general morphology of male genitalia in representatives of the suborders (A) Eupnoi and (B) Laniatores. Although the *pars basalis* (*Pb*) are a long shaft in both suborders, the *pars distalis* (*Pd*), especially the glans (*g*), is more complex in Laniatores than in Eupnoi. (C) Detail of the *pars distalis* in Laniatores (Biantidae) showing the *capsula interna* (*ci*) and *capsula externa* (*ce*). Pushed by hydraulic pressure, the *capsula interna* is everted exposing the conductors (*co*) and the *stylus* (*s*): (D) lateral view; (E) frontal view. (F) Lateral view of the *pars distalis* of another Laniatores (Assamiidae) showing the *stylus* retracted inside the *capsula externa*. With the eversion of the *follis* (*Fo*), also promoted by hydraulic pressure, the *stylus* is exposed: (G) lateral view; (H) frontal view.

ejaculatory duct by a muscular propulsive organ located at the base of the penis; this organ is absent in Cyphophthalmi (Kästner 1935).

The ovipositor in Cyphophthalmi and most Eupnoi has a shaft (sometimes as long as or longer than the female body) composed of cuticular rings connected by segmentally arranged muscles (figures 13.5A–C). It ends in paired bilateral processes derived from one or more rings, and the genital opening is located basally between the these processes (figures 13.3a–c, 13.5A,C). Each process generally bears a tuft of sense organs on the latero-subdistal surface (figures 13.3a–c, 13.5A,C). A pair of seminal receptacles (sclerotized vaginal diverticula) is found inside the genital opening, sometimes associated with glands (de Graaf 1882; Martens 1986) (figure 13.5C). Lack of seminal receptacles is often associated with parthenogenesis (Shultz & Pinto-da-Rocha 2007). The ovipositors

of Dyspnoi and Laniatores differ markedly from those of the other suborders (figures 13.3d–h). Their ovipositors are always much shorter than the female body. Additionally, their segmentation is absent or vestigial, a system of circular muscles surrounds the vagina (except in Trogluloidea), and vaginal symmetry is biradial (rather than simply bilateral); only in the Laniatores the lumen is X-shaped in cross section (Martens 1986). In contrast to the Eupnoi and Cyphophthalmi, Dyspnoi and Laniatores have one or more sperm receptacles in each of their four lobes, and these receptacles are much shorter (figures 13.5D–F). The end of the ovipositor in Dyspnoi has a pair of valves (figures 13.3d,e), but ends in four lobes in the majority of Laniatores (figures 13.3f–h, 13.5E).

As mentioned in chapter 1, Darwin's original distinction between primary and secondary sexual characters has been blurred by findings in the last



**FIGURE 13.5** Schemes contrasting the morphology of ovipositors in representatives of the suborders (A) Cyphophthalmi (*Purcellia* from Hansen & Soerensen 1904), (C) Eupnoi (*Pseudoballarra* from Hunt & Cokendolpher 1991), and (E) Laniatores (*Bishoppella laciniosa* from Martens et al. 1981). Note that the ovipositor of Cyphophthalmi and Eupnoi bears bilateral processes (bp) and tufts of sense organs (so). (B) Longitudinal cut of the distal part of the *truncus* showing the highly sclerotized cuticular rings (cr), the ring folder (rf), and the segmentally arranged longitudinal muscles (lm) (Martens et al. 1981). (D, F) Transversal cuts showing the X-shaped vagina (va), the radial disposition of the seminal receptacles (sr), and circular muscles (cm). All figures redrawn.

few decades on the action of sexual selection (be it by male–male competition or female choice) on the morphology, physiology, and behavior of male and female genitalia. Thus, it is impossible to discuss the evolution of “primary” sexual characters without reference to sexual selection, “secondary” characters, and mating strategies. In order to follow the scope of this book, however, the *characters* in this *play* will be the male and female genitalia, and even their somatic complements (the male and female bodies) will be somewhat seen as their evolutionary context, *the stage*.

Harvestmen are a particularly interesting system to study evolution of sexual characters because they have evolved an intromittent organ independently of other better-studied taxa. Moreover, the penis

and ovipositor in harvestmen are among the largest and most accessible genitalic structures known among terrestrial arthropods and offer exceptional opportunities for exploring the evolutionary factors driving their diversification. The taxonomy of harvestmen at all levels is based largely on the configuration of the penis. The male genitalia of virtually all families has been described at least for one species, and in many cases for several. Nevertheless, no evolutionary, functional or behavioral hypotheses have been put forward to explain the great morphological diversity of male genitalia, and females have been largely ignored due to a widespread assumption that they show little useful diversity. From the standpoint of an evolutionary biologist, this situation is lamentable, but typical

for many groups of terrestrial arthropods: genital diversity is tapped for its taxonomic information and presented only as illustrations in various publications, where they rest like fossils buried in vast strata of paper. We will attempt to establish the relationship between this vast material and the little behavioral data available. Given the few but important hard facts we do know, the following sections present our hypotheses on the macro-evolutionary pathways of genitalia in Opiliones. Since eversible genitalia is an autapomorphic trait of Opiliones, and since Cyphophthalmi presents a spermatopositor instead of a penis, it is difficult to reconstruct the plesiomorphic states both at the basal node of the order and at the basal node of Phalangida, the clade formed by Eupnoi, Dyspnoi, and Laniatores (figure 13.2). However, the hypotheses presented here make the underlining assumption that the genitalic traits observed in Cyphophthalmi represent the plesiomorphic condition for the order.

#### Act I: An Almost Intromittent Spermatopositor and a Sensitive Ovipositor

Sperm transfer by means of a spermatophore is probably a basal trait within Opiliones, and within Arachnida (Proctor 1998). Although only a few spermatophores have actually been recorded, the shape of the male genitalia in all Cyphophthalmi suggests it is the rule in this suborder. Spermatophores have been found attached to the female in a way that suggests direct participation of the male. The spermatophore duct is glued to the tip of the ovipositor near the opening of the sperm receptacles (Karaman 2005). The female lacks a genital operculum and the ovipositor is covered with numerous setae, most likely sensitive to micro-conditions of potential oviposition sites (Machado & Macías-Ordóñez 2007). The spermatophore, on the other hand, is a complex structure in which functional sperm is covered by a layer of modified spermatogonia (Alberti 2005).

It is likely that the male produces the spermatophore while interacting with the female, attaches it to her ovipositor by the spermatophore duct using the spermatopositor, and remains there until it solidifies and/or sperm is transferred. Schwendinger & Giribet (2005) reported a male “copulating” with a female in *Fangensis lecrerci* (Stylocellidae), “belly to belly”, facing in opposite directions, and taping her anal region with his pedipalps, although

no intromission was reported. It is likely that this was a form of pre- or post-spermatoposition courtship, and or mate guarding. Furthermore, this position may also give the female access to the glandular organs in the anal crown and hind legs of the male. As will be detailed below, genital nuptial feeding has been very recently described in Eupnoi, and it may be common in harvestmen. Its origin in male courtship in Cyphophthalmi is a hypothesis worthy of further exploration.

#### Act II: A Daring Penis Appears on the Scene

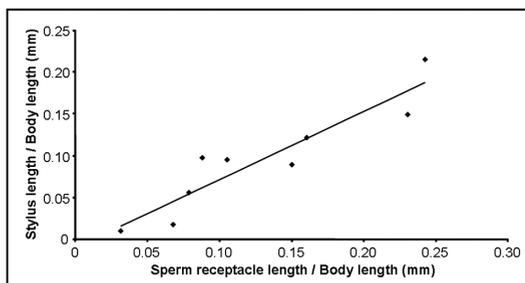
The ovipositor of most of the Eupnoi is similar to that of Cyphophthalmi (figures 13.3a–c). Although the ovipositor of most Acropsopilioninae (Caddidae) is short and has only a few segments, its architecture is basically similar to that of other Eupnoi. The male genitalia, however, is strikingly different from the Cyphophthalmi. Without any known intermediate states, an intromittent penis appears in the Phalangida. All known species have it and the oldest fossil record of any intromittent organ is that of the Devonian harvestman *Eophalangium sheari* (Eupnoi) from the Rhynie Chert, Scotland (Dunlop et al. 2003). If intromittent genitalia evolved from a Cyphophthalmi-like state, the evolutionary leap from such a spermatopositor to a penis is not hard to imagine. Direct sperm transfer by means of a penis may have enabled males to increase sperm production by eliminating the material investment in infertile sperm and protective layers of the spermatophore (Machado & Macías-Ordóñez 2007). Genitalic intromission is the rule in the Phalangida, but based on the morphological and behavioral evidence available, there is a great diversity of ways in which male and female genitalia interact.

There are no detailed descriptions of genitalic interaction published for Eupnoi (or any other suborder). Unpublished studies by J. W. Shultz & R. Macías-Ordóñez, in which intense transmitted light was used to reveal internal copulatory events in *Leiobunum verrucosum* (Sclerosomatidae), show that the penis' *stylus* does not go beyond the first third of the ovipositor, where the openings of the sperm receptacles are located, even when the female is immobilized (see Act IV). Thus, although most of the penis shaft enters the female body in *L. verrucosum* and other Eupnoi, it may not penetrate far inside the female reproductive tract. Since the ovipositor is retracted and may bend inside the

female, the length of the penis inserted beyond the female's genital operculum does not indicate how far it goes into the ovipositor. This is true for all Opiliones since, unlike other groups with internal fertilization, such as insects or mammals, the entrance to the female reproductive tract is not continuous with the apparent external genital opening. Thus, once inside the females' pregenital chamber, the penis must reach the tip of the ovipositor to enter the female reproductive tract.

Once inside the ovipositor, the shape of the *stylus* seems appropriate to enter the sperm receptacles after going through the ovipositor atrium. Furthermore, the *stylus* (and sometimes the glans) of most Eupnoi has an angle so that it would find the opening to the sperm receptacles and bend when entering them, where it could simply deposit the aflagellate sperm. This reasoning predicts that *stylus* length should be positively correlated to the depth of the sperm receptacles. In figure 13.6 we present original data from nine species of the family Neopilionidae showing such a correlation. Although we controlled for body size, we did not control for possible phylogenetic effects because no generally accepted phylogeny of the family is available.

Another remarkable feature of the Eupnoi penis is a morphologically diverse set of structures (sacs, bulbs, and alae) found at the distal end of the trunk. If the *stylus* was inserted in the sperm receptacles, these structures would fall just outside of the ovipositor, near its tip, potentially in contact with the abundant sensilla found on it. A likely possibility is



**FIGURE 13.6** Positive and significant relation between *stylus* length and sperm receptacle length in nine species of the family Neopilionidae ( $y = 0.813x - 0.010$ ;  $R^2 = 0.861$ ;  $p < 0.001$ ). The length of the structures was divided by body length in order to control for the effect to the size. Data taken from drawings presented in Hunt & Cokendolpher (1991) (figures 5d, f; 8b, d; 12d, e; 14b, d; 17b, d; 18b, d; 20f, g; 22c, f; 24a, c).

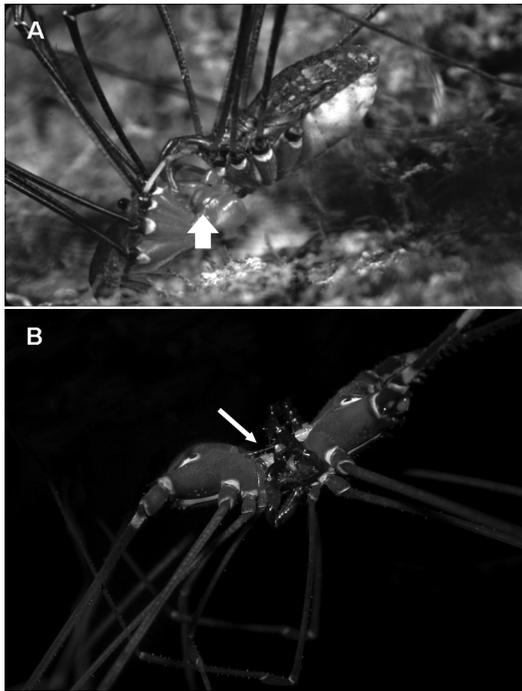
that males stimulate these sensilla while accessing the sperm receptacle as a form of copulatory courtship subject to cryptic female choice (Eberhard 1985, 1996). An alternative view, further discussed below, is that males may be exploiting a female sensory bias, by seductively stimulating the ovipositor's sensilla, used by the female to probe optimal sites for egg laying (Machado & Macías-Ordóñez 2007).

Other features of the male genitalia may be relevant to copulation. Several groups within the superfamily Phalangioidea have glands that open at the base of the non-sclerotized section of the everted genitalia. The sacs, bulbs, and alae of the shaft may also serve as "buckets" that convey secretions from the glands to the female. Suggestive evidence of nuptial feeding prior to copulation by means of penis intromission into the female's mouth has recently been reported for the sclerosomatid *L. verrucosum* (Shultz 2005) and the phalangiid *Phalangium opilio* (Willemart et al. 2006). Moreover, unpublished observations by R. Macías-Ordóñez & J. W. Shultz of the sclerosomatid *Leiobunum vittatum* indicate that the female apparently feeds from the base of the male penis during male grasping (figure 13.7A).

### Act III: A Demanding Ovipositor Appears on the Scene

The penis of Dyspnoi has the same general ground plan of that found in Eupnoi. The ovipositor, however, is strikingly different from that of Cyphophthalmi and Eupnoi. It is shorter and cuticular segmentation has been lost, although segmental muscles have been retained in some Trogluloidea (Martens 1986). Sperm receptacles are smaller and highly variable in number, ranging from one to multiple sacs on each side. In Ischyropsalididae, there are four to ten tube-shaped sperm receptacles, and the number of sensilla at the apex of the ovipositor is greatly reduced (Gruber 2007).

There are detailed records of copulation for two species of the genus *Ischyropsalis* (Martens 1969), showing a few short intromissions (a couple of seconds long) while the female grasps and presumably feeds on the male's cheliceral glands. Although penis microstructures in Dyspnoi have been reported for many species of the seven families that comprise the suborder (see Gruber 2007), detailed genitalic interaction during copulation has never been described. It is evident, however, that the



**FIGURE 13.7** (A) Female of *Leiobunum vitattum* (Sclerosomatidae, Eupnoi) manipulating the inflated hematomochia of the male using her chelicerae (arrow), probably obtaining a nuptial secretion (photo by Joseph Warfel). (B) Face-to-face copulation in *Acutisoma proximum* (Gonyleptidae, Laniatores). Note that the male grasps the pedipalps of the female using his own pedipalps (photo by Bruno A. Buzatto). The arrow indicates the *truncus* of the penis.

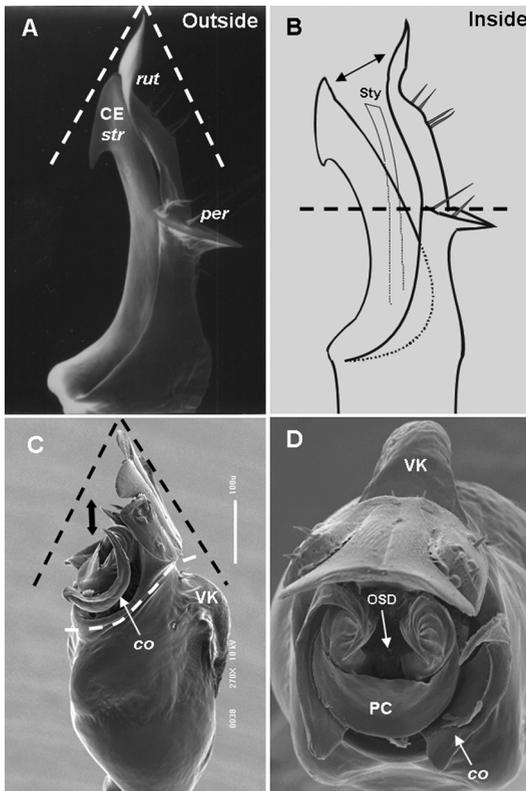
Eupnoi-like penis with its *stylus* does not seem to fit this kind of sperm receptacle. The penis likely leaves the sperm in the lumen of the ovipositor, where the female may have more control of its fate than in Eupnoi. Furthermore, the sensitivity of the ovipositor to the stimulation of the penis may be reduced due to fewer sensilla. However, given the available information, we do not have even a plausible hypothesis on the way the penis and the ovipositor interact in this suborder.

#### Act IV: A Demanding Ovipositor, a Resourceful Penis

The ovipositor of Laniatores is similar to that of Dyspnoi, but with a narrower atrium and a cross like lumen (figures 13.5D, F). The lumen of the

vagina is X-shaped in cross section; the sperm receptacles are radially oriented, varying widely in position and number (in multipliers of four) (Martens 1986) (figures 13.5D, F). The internal morphology of the ovipositor needs to be better studied in the Travunioidea and in Triaenonychoidea (Giribet & Kury 2007). The design of the penis in Laniatores is clearly different from that of previous groups. It is shorter, not heavily sclerotized, and the morphology of the *pars distalis* is highly variable, particularly the glans, where a set of new sclerites results in many different sizes and forms of the *capsula interna* and *externa*. In the incredibly complex male genitalia of the Fissiphalliidae, for instance, the *capsula externa* bears a ventral plate modified in two tagmata: a rounded *pergula* and a spade-like *rutrum* (Pinto-da-Rocha 2007) (figures 13.8A, B). The *capsula externa* in this family also exhibits modifications, such as a rigid *stragulum*, which is articulated to the *truncus* like a jack-knife (Pinto-da-Rocha 2007) (figures 13.8A, B). In Escadabiidae, the *capsula interna* is very wide and can bear modified structures called conductors (Kury & Pérez-González 2007), which are generally sclerotized, blade-like plates located dorso-laterally at the *stylus* (figures 13.8C, D; see also figures 13.4C–E).

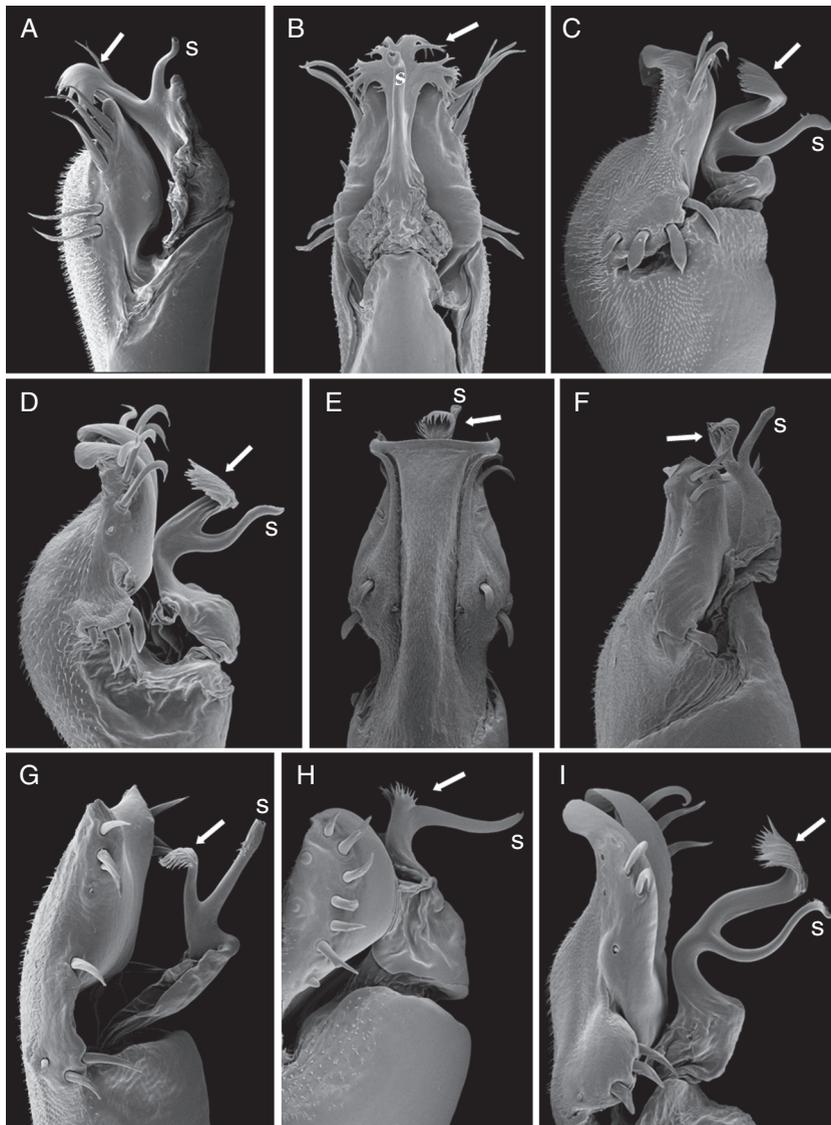
Even though the modified structures of the *capsula interna* and *externa* show very different arrangements among the families of Laniatores (see Martens 1976, 1986), they seem to play three main roles. First, they attach the *pars distalis* of the penis at the distal end of the ovipositor where the seminal receptacles are located (figure 13.5F). Probably some of the strong setae and sensillae perform this function. Moreover, the morphology of the *pergula* suggests that this structure hampers a deep intromission inside the ovipositor (figure 13.8A, B); the same may be the case of the ventral keel in the *pars distalis* of escadabiids (figures 13.8C, D). Second, the modified structures of the *capsula interna* and *externa* seem to promote penetration of the penis in the ovipositor, as in the acute, spade-like profile of the *stragulum* and *rutrum* of the fissiphalliids (figure 13.8A), also observed in the profile of the *pars distalis* in escadabiids (figure 13.8C). Third, they may open the narrow X-shaped vagina, thus allowing sperm deposition inside the lumen. Some examples are the *stragulum* in fissiphalliids (figure 13.8B), the blade-like conductors in some biantids (figure 13.4E), or even the spiny *follicis* in assamiids (figures 13.4F–H). Once inside the



**FIGURE 13.8** (A) Lateral view of the *pars distalis* in the male genitalia of the Fissiphalliidae *Fissiphallius martensi*. The stragalum (*str*) and the blade-like rutrum (*rut*) probably facilitate the penetration in the female reproductive tract when they are closed. The pergula (*per*), on the other hand, is likely to hamper a deep intromission inside the ovipositor. (B) Once inside the narrow lumen of the ovipositor (vagina), the stragalum opens by hydraulic pressure and expose the stylus, which will release sperm in the ovipositor lumen. The dark dashed line shows the putative limit of the penis intromission. (C) Lateral view of the *pars distalis* in the male genitalia of the Escadabiidae *Baculigerus* sp. showing its acuminate profile. In this species, a deep intromission inside the ovipositor is probably prevented by a ventral keel (VK), and the white dashed line shows the putative limit of the penis intromission. The dark arrow indicates that the capsula interna bearing the opening of the sperm duct (OSD) is everted by hydraulic pressure. (D) The parastylar collar (PC) and probably a pair of conductors (*co*) help to open space inside the lumen of the ovipositor.

narrow lumen of the ovipositor (vagina), these structures may open or be everted by hydraulic pressure and expose the stylus, which will release sperm in the lumen. Like in the Dyspnoi, the vagina of the Laniatores has a set of ringed muscles that allow constriction of the lumen. Thus, the sperm deposited in the lumen may fill up the multiple sperm receptacles when it closes, probably pressed by the circular muscles, when the penis retracts. Such a muscular system may also enable females to reject sperm if the entrance to the sperm receptacles may be obstructed. This hypothesis, however, has yet to be tested in future studies. Although the ovipositors of Laniatores show fewer sensilla as compared to the other suborders, the male genitalia possess a set of more proximal and highly variable and ornamented structures (unlike anything else in the other suborders) that seem in an ideal position to stimulate these sensilla. The females' sensilla invaginate when the penis enters, thus maintaining contact with the ornaments of the penis. In contrast to the Eupnoi, in which the male constantly moves the body and penis during intromission, in the gonyleptid *Acutisoma proximum*, for instance, males are motionless during copulation (Buzatto & Machado 2008) (figure 13.7B). We speculate that whereas Eupnoi males find the opening of the seminal receptacle in order to release sperm, Laniatores males leave their sperm in the lumen, and it enters passively into multiple sperm receptacles.

No detailed description of genitalic interaction between male and female in Laniatores exists, but morphological evidence suggests that the penis may not venture far inside the female reproductive tract. In some families, such as Escadabiidae, the sclerotized base of the *pars distalis* is wider than the ovipositor, thus making the penetration below the penis tip impossible, in which case sperm deposition would be restricted to the apical section of the ovipositor. This may have resulted in male genitalic structures capable of removing sperm, as has been described in many other arthropod groups with intromittent genitalia (see references in Kamimura 2000). A handful of species in six distantly related subfamilies of Gonyleptidae have evolved a structure that could serve that function, the ventral process (figure 13.9). This structure has probably evolved independently at least in some of these subfamilies, based on their phylogenetic relationships (Pinto-da-Rocha 2002; Kury & Pinto-da-Rocha 2007). Although no information is available on the actual role of the ventral process



**FIGURE 13.9** Male genitalia showing the ventral process (arrow) in representatives of six non-closely related subfamilies: (A) *Bourguyia* sp. and (B) *Asarcus ingenuus* (Bourguyiinae); (C) *Acrogonyleptes unus* (Hernandariinae); (D) *Geraecormobius nanus* (Gonyleptinae); (E) *Promitobates hatschbachi* and (F) *P. ornatus* (Mitobatinae); (G) *Discocyrtus testudineus* and (H) *Metagyndes pulchella* (Pachylinae); and (I) *Stygnobates barbiellinii* (Sodreaninae). Note that the shape and relative position of the ventral process in relation to the stylus (*s*) is remarkably similar among these subfamilies. The ventral part of the penis is always at the left side, except in (E), which is a ventral view of the genitalia.

during intromission, its shape and relative position is remarkably similar among these subfamilies and suggests that it may penetrate the lumen of the ovipositor along with the stylus, smoothly “brushing” the inner walls on the way in, but scraping off

the same surface on the way out. Both the stylus and the ventral process are hardened by hydraulic pressure, thus potentially giving the male some extra control to maneuver these structures inside the female ovipositor (vagina). The process of

sperm removal may explain why copulation in gonyleptids may last up to 20 min in some species (B. A. Buzatto & G. Machado, unpublished data).

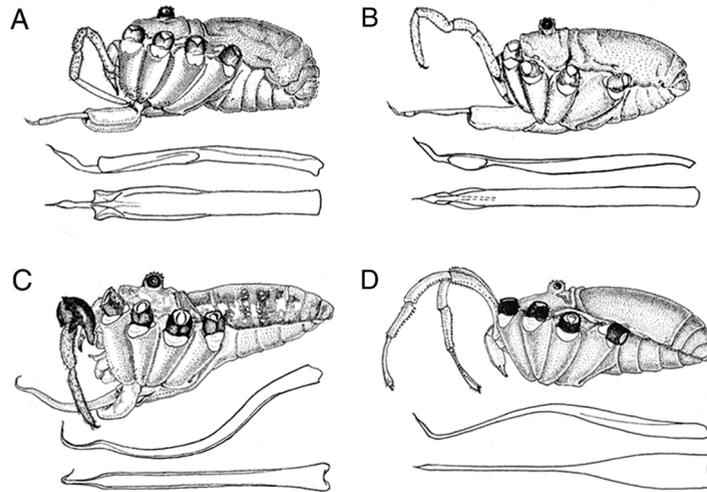
A MICRO-EVOLUTIONARY  
SCENARIO: EVOLUTION OF  
MALE GENITALIC DIVERSITY IN  
*LEIOBUNUM*

Male Genitalic Diversity

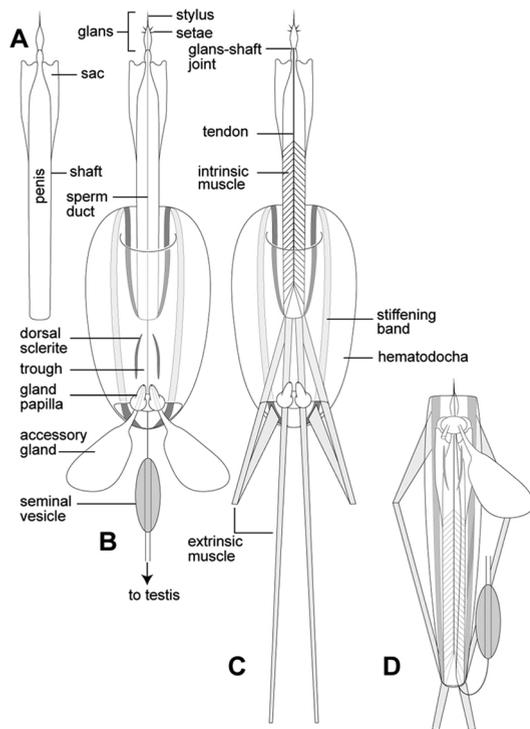
The subfamily Leioibuninae (Sclerosomatidae, Eupnoi) has an essentially Holarctic distribution with major centers of known diversity in North America (especially the Appalachian Region and southeastern U.S.A.), Mesoamerica, East Asia, and the Europe-Mediterranean Region (e.g., Suzuki 1976; Martens 1978; Cokendolpher & Lee 1993). In this section male genitalic diversity in American leioibunine harvestmen is briefly summarized, some details of mating behavior are reported for the first time, and testable hypotheses—based on observations of genitalia in action—are proposed. We focus on male genitalic diversity within leioibunines of eastern North America (*Leiobumum*, *Nelima*, *Hadrobunus*, *Eumesosoma*), with about 30 species. The male genital apparatus comprises a tubular,

sclerotized penis and basal, membranous hematochocha (figures 13.10 to 13.12). The penis has a long shaft that articulates with a short terminal glans, with the tendon of an intrinsic penial muscle operating the shaft–glans joint. The hematochocha is a bag of flexible cuticle that is inflated under hemolymph pressure during mating. The hematochocha acts as a flexible turret for the everted penis and forms the walls of the pregenital chamber when the genital apparatus is withdrawn (figures 13.10 and 13.11).

Penial diversity has been categorized into two broad and probably non-monophyletic groups—sacculate and lanceolate (McGhee 1970, 1977)—based on subterminal modifications of the shaft (figures 13.10 and 13.11). The sacculate condition is plesiomorphic based on outgroup comparisons, with the subterminal apparatus consisting of bilaterally paired, distally opened, chitinous sacs (figures 13.10A and 13.11A). In the inverted state, a pair of glandular papillae at the base of the hematochocha enters the openings to the chitinous sacs (figure 13.11D). The lanceolate condition encompasses a diverse assemblage of penis types united by the absence of sacs. Four North American lanceolate groups have been recognized thus far, the *L. calcar* (figure 13.10C), *L. vittatum* (figure 13.10D), *L. formosum* and *Hadrobunus* species groups, as well as *L. holtae* (J. W. Shultz, unpublished data).



**FIGURE 13.10** Representative males from four *Leiobumum* species illustrating genitalic diversity. Each figure depicts a lateral view of a male and enlarged lateral and dorsal views of the penis. (A) *L. aldrichi* with sacculate penis. (B) *L. politum* with bulbate penis. (C) *L. calcar* with lanceolate penis and robust, clasp pedipalps. (D) *L. speciosum* with lanceolate penis and elongate, spiny pedipalps. All figures after Bishop (1949).

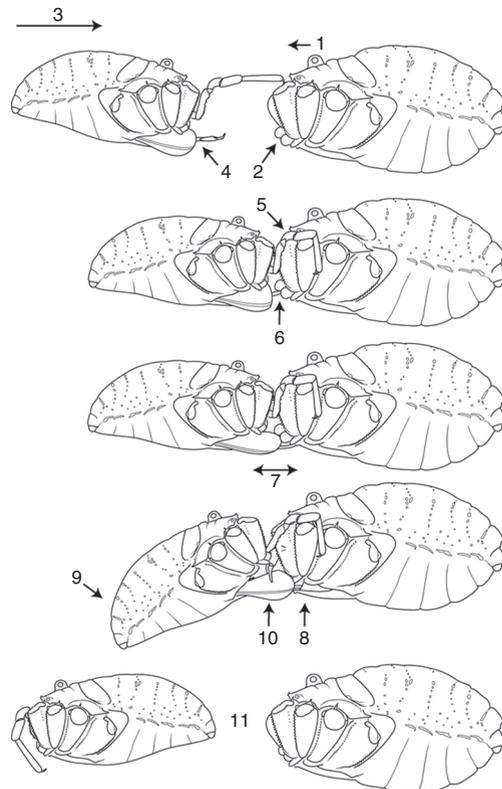


**FIGURE 13.11** Diagrammatic dorsal views of the genital apparatus of the primitively sacculate species, *Leiobunum verrucosum*. (A) Penis. The sclerotized portion of the genital apparatus and the only part traditionally described by systematists. (B) Everted genital apparatus emphasizing the cuticular elements, genital tract and accessory glands. (C) Everted genital apparatus emphasizing cuticular elements and muscles. (D) Inverted genital apparatus. Note that the gland papillae are inserted into the penial sacs.

#### Mating Behavior in a Primitively Sacculate Species

In an attempt to understand the broader significance of penial diversity in leiobunines, Shultz (2005) examined mating behavior in several *Leiobunum* species but focused on a primitively sacculate form, *L. verrucosum*—formerly *L. nigripes* (Shultz 2008). Mating in ten virgin pairs was recorded. In five cases, the female was immobilized by a stick glued to her dorsum to allow detailed inspection of male–female interactions. The latter treatment did not appear to affect the order of mating events, although the male was more likely to mate multiple times as the female could not adopt the “face down” rejection posture (Macías-Ordóñez 2000).

The sequence of events is summarized in figure 13.12 and the following numbers correspond to those in the figure. Upon contact with a male, (1) a receptive female oriented to the male and (2) opened her stomotheca (“mouth”) by inflating the membranous portion of the coxapophyses (“lips”) of the pedipalps and leg I. Unreceptive females faced the substratum and/or turned away from the male. (3) The male moved rapidly toward the female, face to face, and (4) everted his penis. (5) The male used his pedipalps to clasp the female behind the coxae of her second pair of legs and (6) inserted his penis rapidly into the female’s stomotheca, removed it and placed it at the opening of the female pre-genital chamber. Flexible walls likely ensure that penial sac contents are deposited in the female’s stomotheca upon withdrawal. Some males did not insert the penis into the female’s stomotheca. (7) A variable interval was spent with the penis tip positioned just within or at the opening of the



**FIGURE 13.12** Summary of events during mating in the sacculate species, *Leiobunum verrucosum*. The events indicated by numbers are described in the text.

pregenital chamber, with the dorsal surface of the hematodocha forming a conduit from basal nuptial glands to the female's mouth. The female manipulated the dorsal surface of male's hematodocha with her chelicerae. (8) After a variable interval (several minutes), the penis gained entry into the female pregenital chamber and (9) the male assumed a "face up" position, exposing the gland papillae at the base of the hematodocha. (10) A variable interval was spent with the female feeding on nuptial secretions and with the male making deep penetrations into the female pregenital chamber, apparently copulating. (11) The male withdrew his penis and departed. No postcopulatory mate guarding was observed nor has been reported in this species. The female sometimes groomed her ovipositor following copulation.

These observations debunk the widespread supposition that *Leiobunum* mate indiscriminately without precopulatory courtship (e.g., Bishop 1949; Edgar 1971), and traces the original error to a remarkable similarity between copulation and the preceding close-contact "courtship" involving the male genitalia. The primitive sacculate genital apparatus apparently serves a dual role as a genital organ and as a delivery mechanism for a male-generated nuptial gift, with the gift being offered in three phases: an initial delivery by the subterminal sacs directly to the female's open stomotheca, a second delivery via a stream of secretion traveling along the dorsum of the hematodocha, and, finally, full exposure of the gland papillae to the female that coincides with the female granting the male access to her pregenital chamber.

#### Mating Behavior in a Derived Lanceolate Species

A long-term field study of *Leiobunum vittatum* in the eastern Pennsylvania (U.S.A.) has revealed that suitable substrate for oviposition is limited to cracks in rocks and fallen logs, which males actively patrol and fight for with other males (Macías-Ordóñez 1997, 2000). Mating pairs and ovipositing females may be found from late August to early November, when the first frosts kill all the adults in the population. When a female encounters a rock, she slowly goes over the whole surface, inserting the ovipositor inside cracks, and probing potential sites before laying one or many eggs. This behavior is impossible when they encounter a male, since on contact the male eagerly attempts to grasp her using his

pedipalps as described above. The female may reject intromission, but grasping seems harder to avoid. If the female escapes grasping, however, she usually must abandon the rock to avoid the male, thus abandoning also the opportunity to find a suitable oviposition substrate. If copulation proceeds, however, a series of short repeated intromissions will take place for a period of a few minutes up to about one hour.

As described in other Eupnoi, between intromissions, the female seems to obtain some sort of "nuptial" secretion from the base of the male genitalia using her chelicera (R. Macías-Ordóñez & J. W. Shultz, unpublished data, figure 13.7A). In fact, the female actively strokes the male around his genital operculum with her pedipalps while his genitalia is not everted seemingly to stimulate genitalic eversion. After close examination, it is clear that most of the times the male everts his genitalia, no intromission takes place but the female always seems to obtain something from the glands at the base of the *truncus*. However, in contrast to *L. verrucosum*, insertion of the penis into the female's stomotheca has never been observed. Once male grasping is over, the male will guard the female by wrapping one or two female legs at the femur or tibia with the terminal tarsi of his own first pair of legs and following her while she walks around. The female seems free to probe the rock at will, undisturbed not only by this male, but by any other male, since the guarding male will aggressively expel any other approaching male. A male will stop guarding in this fashion only when the female abandons the territory. Thus, by accepting copulation a female gains the opportunity to have full and harassment-free access to the scarce rock cracks where the eggs may safely spend the winter. When the end of the short reproductive season is near, females may encounter and copulate with two or three males within a few hours in their search for oviposition substrates. The protection offered by a resident male may be worth taking and may imply yet stronger selection for cryptic mechanisms to influence the paternity of her eggs (Machado & Macías-Ordóñez 2007).

#### What Factors Could be Driving Male Genital Diversity in *Leiobunum*?

The loss of penial sacs in several leiobunine groups may represent elimination of the first phase of a primitive gift-delivery sequence. Because sac loss is

typically associated with novel clasping mechanisms in the male pedipalp (see figures 13.10C, D), the appearance of lanceolate penes may reflect an evolutionary movement along the strategic spectrum from female enticement toward coercion. Significantly, in species in which male anatomy has departed most from the primitive condition (e.g., *L. calcar* and *L. speciosum*), females show modifications of the pregenital opening that appear to either exclude or entrap the penis, suggesting that the lanceolate male strategy has promoted evolutionary responses in female morphology. These emerging trends are consistent with a scenario in which male genitalic innovation is driven by sexual conflict, resulting in a possible sexual arms race. In this race, females would have an advantage if they are able to exert cryptic choice after receiving nutritional gifts and or harassment protection by their mates.

It is important to note that the lanceolate penis in *Leiobunum* appears to be a phenomenon limited primarily to temperate regions; virtually all tropical species examined thus far are sacculate (J. W. Shultz, unpublished data). Furthermore, within the temperate region sacculate species occur in the milder southern regions or, farther north, overwinter as subadults and gain sexual maturity in late spring. There appears to be only one exception (i.e., *L. aldrichi*) (figure 13.10A), which matures in summer. In contrast, lanceolate species attain sexual maturity in mid to late summer or even later. The theme emerging from these observations is that sacculate species prosper in regions with long reproductive seasons, during which males and females can gather resources for gifts and eggs, respectively. In climate/life-history combinations where young adults have a high probability of abundant future resources, the fitness value of the nuptial gift to the female may be minor compared to that of resources she can gather herself, and the cost of losing a small gift to an unreceptive female may be relatively inexpensive to the male. However, as the duration of resource availability for egg and gift production decreases, the value of the nuptial gift to the female and its potential costs to the male may increase. Under these conditions, male gifts may have a greater impact on the total number of eggs the female can produce. For the male, the cost of giving limited gifts to an unreceptive female may be high, thus leading selection to favor the elimination of the sac-borne initial gift in favor of mechanisms that increase the probability of a *quid pro quo*

exchange of gift and copulation, as in the case of *L. vittatum*. If this scenario is correct, then it would appear to represent a case where male genital diversification has been driven to a large extent by natural selection for efficient use of nutritional resources. Research devoted to testing these proposals is ongoing.

## HYPOTHESES OF GENITALIC EVOLUTION

### Origin Versus Current Function of Sexual Characters

Any trait may appear and then continue to perform its original function, or it may perform functions completely different from those that provided its initial selective advantage. It is hard to talk about origin without a robust phylogeny in which characters are mapped and ancestral states are identified. Inferring the relative phylogenetic timing of origin, however, does not necessarily reveal original, function although correlation with other traits may help to infer such ancestral functions. Function, however, may also be simply defined as “what the trait does” without any assessment of its contribution to fitness (proximate function). Alternatively, function may imply “how a trait contributes to fitness”, i.e., the ultimate function (see discussion in Coddington 1988). From an evolutionary standpoint, maintenance of the trait must be discussed based on current function, but only if “function” is defined as contribution to fitness.

Evolutionary biologists frequently address the potential fitness advantage of a trait by making several assumptions about actual functional morphology, which are rarely corroborated. This has often been the case when debating “function” of genitalic traits (e.g., Eberhard 2004b), most likely because the taxonomic literature provides abundant information on genital morphology, but none on how it works. Such is the case for genitalia in Opiliones. Furthermore, the most useful structures in taxonomy are sclerotized, but soft parts are frequently not depicted. When they are depicted, they are frequently collapsed by preservation or in a resting position, at best. Given that the base of the genitalia in Opiliones usually has a strong hydraulic component, we lack even the morphological information to infer proximate function of these structures. Thus we have been forced, as most

everyone else, to speculate on function based on a small data set of behavioral observations, and a larger base of morphological information. It is our hope that such speculation may stimulate empirical research both on proximate function and fitness consequences of genital morphology in this group.

Our main hypothesis for the origin and initial function of the male genitalia is that it appeared as a spermatophore placement structure (a spermatopositor) in the Cyphophthalmi, and then evolved into an intromittent organ (a penis), a function retained in the rest of the suborders. Insemination seems to range from dynamic in Eupnoi to passive in Laniatores. While insemination in Eupnoi seems to demand intense movement of the penis due to a long, highly mobile and flexible ovipositor with a single entrance to a single pair of sperm receptacles, a shorter and less flexible ovipositor with multiple small receptacles in Dyspnoi and Laniatores suggests a more passive copula, as insemination requires simply opening the ovipositor and leaving sperm in the lumen.

Besides taking male gametes closer to female gametes, it seems reasonable that, as it is the case in other animal groups (examples in Eberhard 1985), the penis has a stimulatory and/or coercive function. In the case of the female ovipositor as a terminal organ of the reproductive tract, it may seem obvious that it appeared as an egg-laying structure, capable of inserting eggs deep in the substrate. The female ovipositor may have incorporated a secondary function as a discriminatory organ in charge of screening male stimulatory performance. Such secondary function is puzzling in the suborders Dyspnoi and Laniatores; ovipositor evolution may have been driven by natural selection acting on egg-laying strategies or by sexual selection as a consequence of an arms race between males and females. The implications of ovipositor reduction in size and sensibility due to sexual selection would be profound. Since short and less sensitive ovipositors would not be able to explore deep crevices and assess proper conditions for egg development, females would lay eggs on the substrate and in some cases remain nearby and brood them. Therefore, cryptic female choice and male sperm competition would have been the forces behind changes in egg-laying structures and, consequently, oviposition and brooding strategies (see discussion in Machado & Macías-Ordóñez 2007).

### Sperm Competition, Female Choice and Sexual Conflict

Genitalic function involves many and diverse morphological, physiological, and behavioral traits. Penes and ovipositors are trait arrays subject simultaneously to selection on the whole array and on single traits or sub-arrays somewhat independently. In Opiliones, as in many other groups, one of the main selective forces on these arrays is the morphology and behavior of the opposite sex during copulation. The relative importance of sperm competition versus cryptic female choice driving the evolution of primary sexual characters has been the focus of some debate (see chapter 1). Sperm competition (intrasexual selection among sperm or other male products from different males inside the female reproductive tract) seems ubiquitous among polyandrous species, and Opiliones are unlikely to be an exception. The selective power of sperm competition on male genitalia is undisputed and may be the source of much evolutionary change in Opiliones. However, sperm competition has never been studied in any harvestman species.

Antagonistic coevolution (*chase-away*) and positive-feedback coevolution (Fisherian *run-away* and good-genes) have also been contrasted in the last decade, as competing models in the evolution of sexual characters (Holland & Rice 1998; Hosken & Stockley 2004). This debate has been fueled by mixing arguments of origin and function. Many male genitalic traits may have imposed fitness costs to females when they appeared, but may currently be under Fisherian selection. Kokko et al. (2003) have suggested that these and other models of sexual selection by female choice are not mutually exclusive and may represent two sides of the same coin, or two stages during the evolution of sexually selected traits. Once the male and the female are interacting inside the female reproductive tract, the female has more control over the fate of the male's gametes, even more so once the male has withdrawn his genitalia (Eberhard 1996). The elaborate and highly diverse male genitalia of the Opiliones could be expected under a process of cryptic female choice in which females select males based on their stimulatory abilities (Machado & Macías-Ordóñez 2007). A sexual conflict chase-away scenario may also be imagined in which males may "seduce" females by stimulating their ovipositors and inducing the female to mate suboptimally

(Arnqvist & Nilsson 2000). These scenarios are impossible to tell apart without accounting for all costs and benefits involved for both sexes, which are likely to be context dependent. Furthermore, the mating systems of some harvestman species are known to differ sharply between populations, probably due to context dependent cost–benefit relations. Male *Leiobunum vittatum*, for instance, defend mating territories and guard females after copulation in eastern Pennsylvania, U.S.A. (Macías-Ordóñez 1997), but not in Michigan (Edgar 1971), 1,000 kilometers away. Different selective pressure by sperm competition, harassment of females by males, and cryptic female choice are most likely operating in each of these populations. Such inter-population differences in the mating system are well documented in other animal groups (Emlen & Oring 1977; Kokko & Rankin 2006).

Genital evolution in harvestmen apparently involved an initial phase of enlargement, then change into an intromittent organ, a general tendency to reduction in ovipositor size and sensitivity, followed by an increase in penis complexity. Given that the entrance to the female reproductive tract is not attached to the genital opening, the penis probably does not go beyond the sperm receptacles, located very near the tip of the ovipositor retracted inside the female body. Male strategies for direct insemination and enhanced female stimulation may have coevolved with female strategies that restrict accessibility to the seminal receptacles by means of ovipositor reduction, loss of sensibility, and even promotion of sperm competition, observed now in the form of male structures that probably remove sperm from previous males from the female reproductive tract (Eberhard 1996; Birkhead & Møller 1998).

The juxtaposition of the genital opening and the mouth, so that the female mouth is close to the base of the male penis, has probably promoted the evolution of “genital nuptial feeding” in Eupnoi, sometimes involving the penis. In such cases, the “primary” sexual organ of Opiliones may be under sexual selection via female choice. Females may also select for size of penial receptacles containing nuptial gifts and or stimulation on sensilla at the tip of their ovipositor. Nuptial gifts in Dyspnoi, however, do not come from the male genitalia, but from different glands located on the chelicerae.

As suggested by Hosken and Stockley (2004), exploring the mechanisms of genital evolution is a

fertile ground to test predictions derived from different sexual selection models. The Eupnoi penis may have originated as a way to impose insemination on the female by getting the gametes closer to the sperm receptacles, and even “seducing” the ovipositor by exploiting a sensory bias on its sensilla. In time, females seem to respond by restricting access to receptacles by means of shorter, constraining and less sensitive ovipositors, thus turning them into penis-screening devices, which now must not only court the ovipositor but even feed the female. It may be common in other taxa that a male trait originates in a sexual conflict context (as suggested in the section of male genital diversity in *Leiobunum*), falling later in a female-screening evolutionary process. Given their abundance, diversity and relatively large genitalia, Opiliones seem to be great candidates to shed light on this and other evolutionary mechanisms. Overall, we hope to motivate colleagues to challenge our hypotheses and pursue research in this fascinating group.

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