Chapter 10

Phylum Nematomorpha

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PHYLUM NEMATOMORPHA

Chapter Outline

INTRODUCTION

Twenty-two species of freshwater horsehair worms (Gordiida) are known from the Nearctic. Together with five marine species from the genus *Nectonema*, Gordiida form the taxon Nematomorpha. They are parasites of insects (mostly grasshoppers, crickets, and beetles) and emerge from these hosts at maturity. The usually terrestrial host is driven towards water, where the horsehair worm is released. Reproduction takes place in the water, where the tiny and morphologically distinct larvae infect paratenic hosts. Although relatively little is known about the gordiid life cycle, the intermediate host (e.g., mosquitos, mayflies) generally spans the gap between the aquatic and the terrestrial environment. Recent reviews were published by Hanelt et al. (2005) and Schmidt-Rhaesa (2012). The Nearctic nematomorphs have been reviewed by Schmidt-Rhaesa et al. (2003).

LIMITATIONS

Sexes, but usually not species, can be distinguished with simple optics (e.g., a magnifying glass). In females, the cloacal opening is always terminal on the rounded posterior end, with the exception of *Paragordius varius*, where the posterior end has three lobes, and the cloacal opening is located between these lobes. In males, the cloacal opening is always subterminal and on the ventral side. Most Nearctic species have two tail lobes posterior of the cloacal opening (*Gordius, Gordionus, Parachordodes*, and *Paragordius*). In contrast, representatives of *Chordodes, Neochordodes*, and *Pseudochordodes* have no tail lobes but still possess a ventral cloacal opening. The genus *Gordius* can be recognized by a semicircular fold, the postcloacal crescent.

Gordiids are sometimes confused with large nematodes from the taxon Mermithida. In premature, whitish stages, these taxa may be indistinguishable. Some hints for a proper distinction are a usually darker color in gordiids, pointed (Mermithida) versus rounded (Gordiida) body ends, the presence of tail lobes (some Gordiida), or the presence of a white anterior tip followed by a dark brown ring (Gordiida) (Fig. 10.1). In addition, mermithids are usually transparent under transmitted light, whereas gordiids will appear opaque due to their thick layering of cuticle.

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FIGURE 10.1 Ventral view on head end of an undetermined gordiid species showing the whitish anterior tip, followed by a darker collar. A dark ventral line extends from the collar along the body.

Proper identification requires higher magnifying techniques. A superficial cuticle sample with attaching musculature removed gives a good impression of the surface structure, especially the pattern of areoles. Nevertheless, scanning electron microscopy (SEM) has become the standard tool for species identification of gordiids, as fine structures can be documented most reliably.

One problem is that the significance of intraspecific morphological variation of characters in gordiids is not well understood. Thus, it is not clear whether minute morphological differences among specimens indicate species differences or are due to intraspecific variation.

Molecular "barcoding," the comparison of fast evolving sequences among specimens, has recently become more common in gordiids (e.g., Begay et al., 2012) and may help identify the numerous cryptic species within this phylum. For example, the common species "*Gordius robustus*" likely represents a large cryptic species complex composed of at least eight distinct genetic lineages (Hanelt et al., 2015) with very few diagnostic characters (e.g., smooth cuticle, absence of bristles). Some further species names are regarded as synonyms (see Schmidt-Rhaesa et al., 2003). For a review on the genus *Gordius*, see Schmidt-Rhaesa (2010).

Not all species of *Gordionus* are well and reliably described (see Schmidt-Rhaesa et al., 2003).

At the parasitic stage, gordiids can usually not be distinguished morphologically. Due to the parasitic lifestyle, adult, free-living specimens are seasonal and rarely found. As gordiid larvae encyst in a variety of paratenic hosts, a search for cysts in squeezed host tissue can provide information on the presence of cysts. At least the larvae of the three most common species of gordiids can be distinguished on the morphology of the encysted larva (see Hanelt & Janovy, 2002; Szmygiel et al., 2014).

TERMINOLOGY AND MORPHOLOGY

Gordiids have comparably few diagnostic characters. In the anterior end, the shape (distinctly tapering or more stout), as well as the coloration, is important. The anterior tip is usually whitish, in several species; this is followed by a dark brown or black ring often called dark collar. A ventral and probably also a dorsal longitudinal line of darker coloration can be present. In the posterior end, tail lobes can be present in females of *Paragordius varius* and in males of *Gordius, Paragordius, Gordionus*, and *Parachordodes* (Fig. 10.2 A). The single cloacal opening in the posterior end leads into the intestinal tract as well as to the gonads. A semicircular fold posterior of the cloacal opening in males is called the postcloacal crescent (Fig. 10.2 C). Other structures in the posterior end of males are cuticular bristles or conical spines. In some species, such structures are clustered in certain regions. Spines are scattered on the inner side of the tail lobes; these are often called the postcloacal spines (Fig. 10.2 A). Bristles can be present on the entire ventral side of the posterior end of the tail lobes or in rows anterolateral of the cloacal opening; these are the precloacal bristlefields (Fig. 10.2 A).

The cuticle is smooth in few species but features regular elevations ("areoles") in others (Fig. 10.3 A–G). Areoles can be present as polygonal structures covering the entire cuticle (Fig. 10.3 A, E–G). They are separated by grooves, in which interareolar structures can be present (Fig. 10.3 G). In *Pseudochordodes*, two different types of areoles are present, with the larger one called a megareole (Fig. 10.3 C, D). In *Chordodes*, elevated areoles ("crown areole") have an apical "crown" of short or long bristles (Fig. 10.3 B).

The larvae are morphologically distinct from adults. They are very small (50–100 µm) and are composed of two body regions, a preseptum and a postseptum (Fig. 10.5 B–E). The preseptum includes rings of hooks and three stylets to penetrate the intestinal wall of the intermediate host (Fig. 10.5 C). A large glandular structure, the pseudointestine, occurs in the postseptum (Fig. 10.5 B–D) and varies structurally among species. Anterior of the pseudointestine, another gland can be present. In the intermediate host, the larvae are coiled inside cysts (Fig. 10.5 A). Larvae can be distinguished by their size, the number of folds within the cyst, and by the structure of the pseudointestine (see Hanelt & Janovy, 2002).

MATERIAL PREPARATION AND PRESERVATION

Nematomorphs are best preserved in 70% ethanol, except for DNA work, for which material should be preserved in 100% ethanol and placed into the freezer. For scanning electron microscopy, follow the usual protocols of dehydration, critical point drying, and sputtering. For light microscopy, cuticular samples can either be removed by a tangential section with a razor blade when the worm is stretched over a finger, or by cutting a complete section from the midbody region. This section (length about 1 mm) should be cut longitudinally and internal tissue scraped off with a scalpel. The cuticle can then be observed on a slide either in ethanol or preferably after transfer into glycerol.

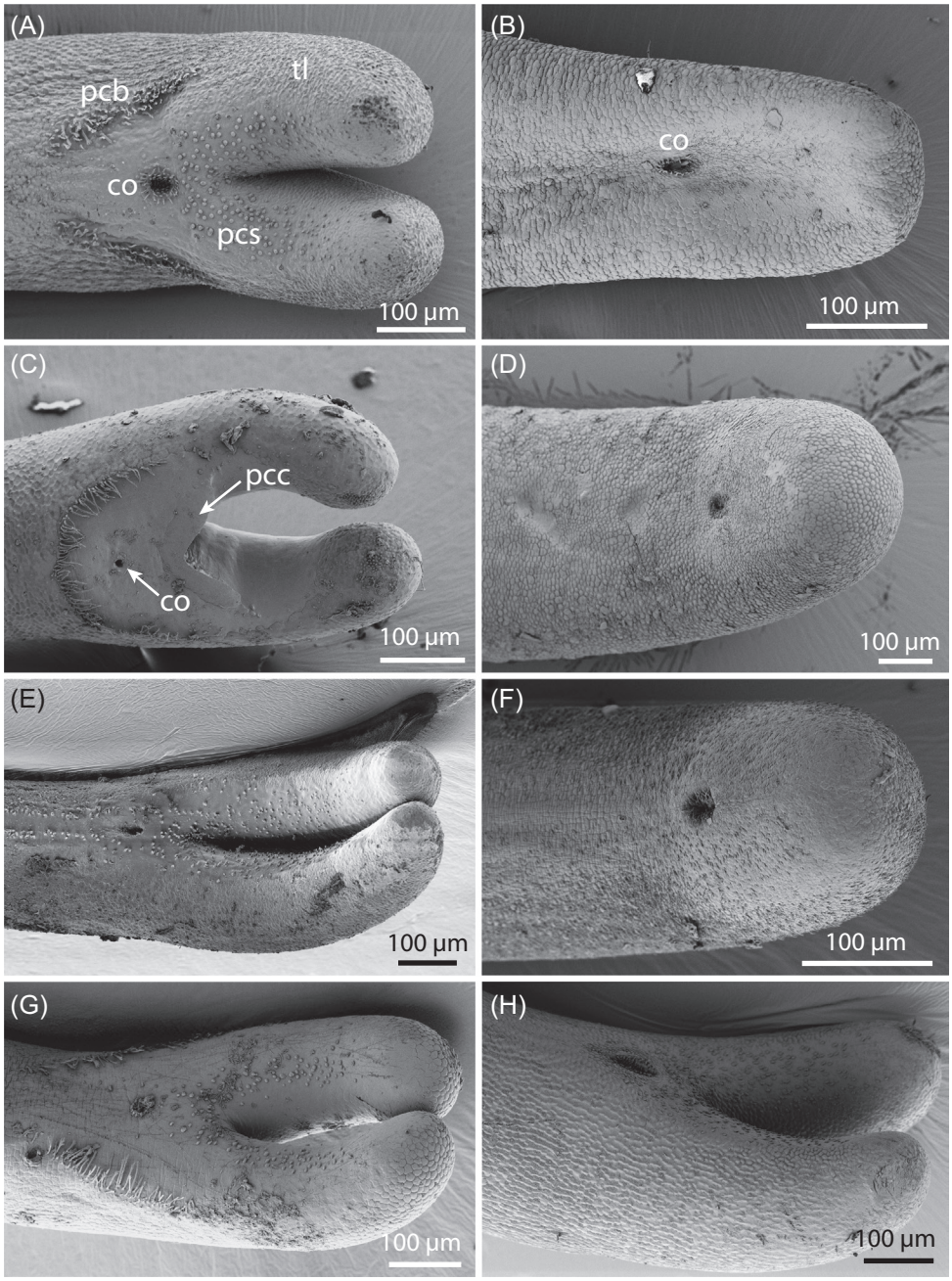

FIGURE 10.2 Ventral side of posterior ends in males. (A) *Gordionus violaceus*. (B) *Neochordodes occidentalis*. (C) *Gordius difficilis*. (D) *Pseudochordodes bulbareolatus*. (E) *Paragordius varius*. (F) *Chordodes morgani*. (G) *Gordionus lineatus*. (H) *Gordionus sinepilosus*. Abbreviations: co = cloacal opening, pcb = precloacal bristles, pcc = postcloacal crescent, pcs = postcloacal spines, tl = tail lobes. All images SEM.

KEYS TO GORDIIDA

Gender

1 Ventral cloacal opening (Fig. 10.2 A–H) ..... male

1' Terminal cloacal opening (Fig. 10.4 B) ..... female

Gordiida Males: Genera

1 Paired tail lobes present (Fig. 10.2 A) 2

1' Tail lobes absent, posterior end rounded (Fig. 10.2 B, D, F) 4

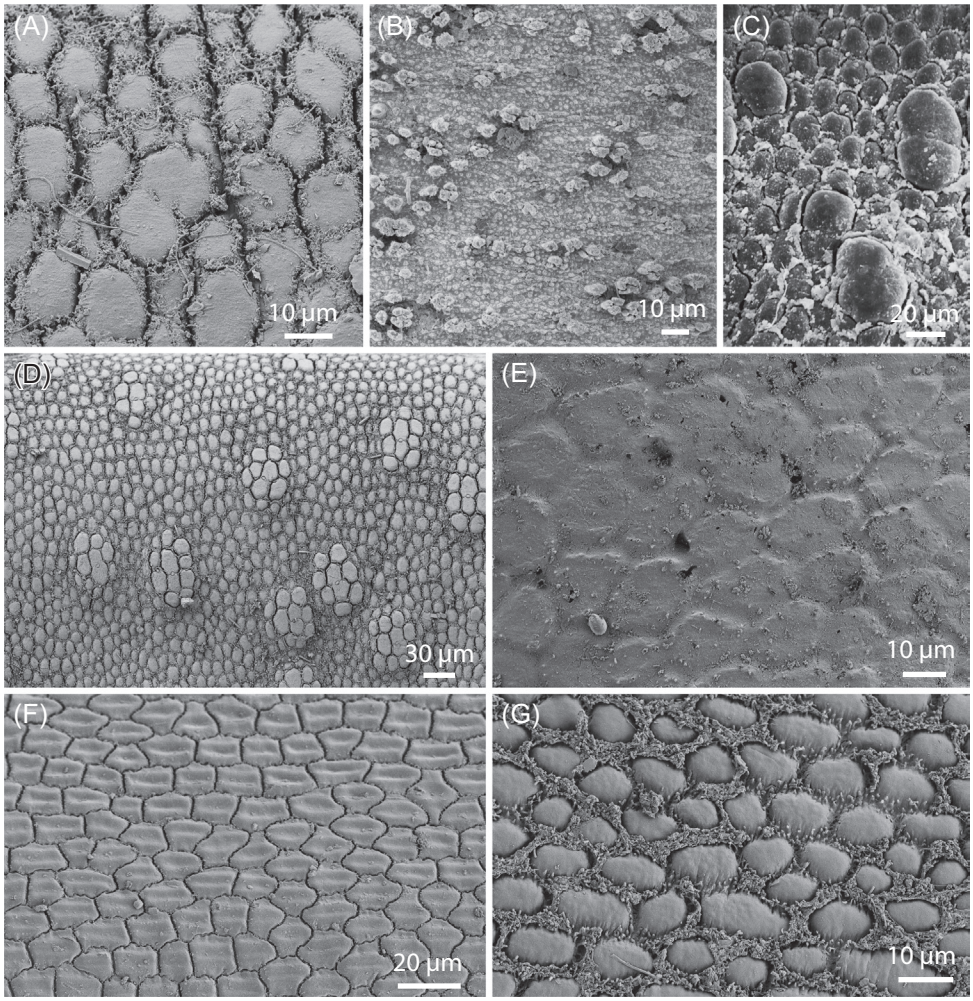

FIGURE 10.3 Areoles on the cuticular surface. (A) *Neochordodes occidentalis*. (B) *Chordodes morgani* with groups of elevated areoles among flatter parts. (C) *Pseudochordodes manteri*, elevated large megareoles among smaller simple areoles. (D) *Pseudochordodes bulbareolatus*, clusters of megareoles bulge out. (E) *Gordius attoni*. (F) *Gordionus lineatus*. No interareolar structures present. (G) *Gordionus violaceus* with interareolar bristles. All images SEM.

2(1)	Postcloacal crescent absent (Fig. 10.2 A)	3
2'	Postcloacal crescent present (Fig. 10.2 C)	***Gordius* [p. 185]**
3(2)	Tail lobes longer than two times the width of one lobe (Fig. 10.2 G)	*Paragordius varius* Leidy, 1851
	[Canada, USA, Mexico]	
3'	Tail lobes shorter than two times the width of one lobe (Fig. 10.2 A, G, H)	***Gordionus* or *Parachordodes* [p. 185]**
4(1)	More than one type of areoles present (Fig. 10.3 B–D)	5
4'	Only one type of areoles present (Fig. 10.3 A)	***Neochordodes* [p. 187]**
5(4)	Crown areoles present (Fig. 10.3 B)	*Chordodes morgani* Montgomery, 1898
	[USA: Florida, Iowa, Indiana, Kentucky, Maryland, Michigan, Missouri, Nebraska, North Carolina, Pennsylvania, South Carolina, Tennessee, Texas, Wisconsin]	
5'	Crown areoles absent (Fig. 10.3 C, D)	***Pseudochordodes* [p. 187]**

Gordiida Females: Genera

1	Posterior end rounded (Fig. 10.4 B)	2
1'	Three tail lobes present (Fig. 10.4 A)	*Paragordius varius* Leidy, 1851
	[Canada, USA, Mexico]	

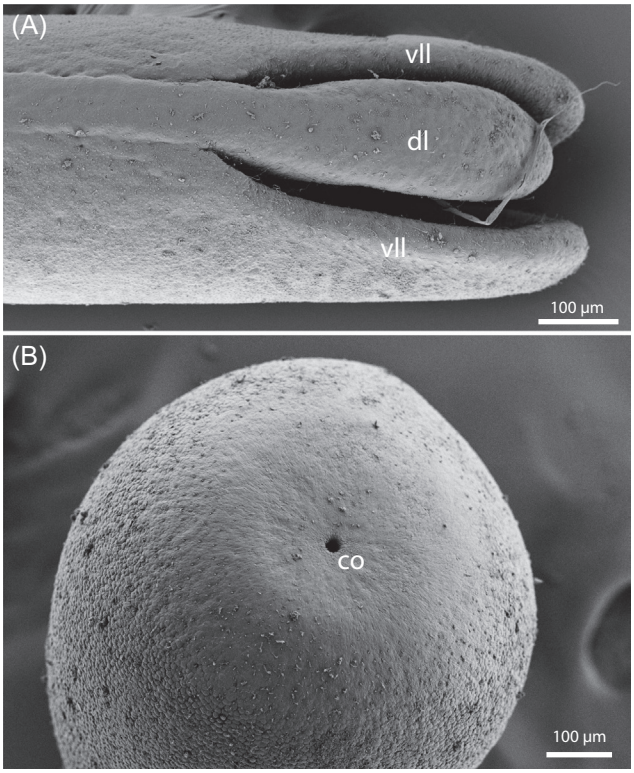

FIGURE 10.4 Posterior end of females. (A) *Paragordius varius* with three lobes, one dorsal (dl) and two larger ventrolateral ones (vll). (B) Round posterior end of a non-Nearctic *Chordodes* female. The cloacal opening (co) is terminal. All images SEM.

2(1) Cuticle with areoles 3

2' Cuticle smooth ..... *Gordius robustus* Leidy, 1851

3(2) Crown areoles absent 4

3' Crown areoles present (Fig. 10.2 C) ..... *Chordodes morgani* Montgomery, 1898

[USA: Florida, Iowa, Indiana, Kentucky, Maryland, Michigan, Missouri, Nebraska, North Carolina, Pennsylvania, South Carolina, Tennessee, Texas, Wisconsin]

4(1) Only one type of areoles present (Fig. 10.3 A, E–G) ..... ***Gordius, Gordionus,*** or ***Neochordodes*** **[p. 187]**

4' More than one type of areoles present (Fig. 10.3 C, D) ..... Pseudochordodes or *Parachordodes tegonotus* Poinar, Rykken & LaBonte, 2004

[USA: Oregon]

Gordiida: Male *Gordius:* Species

1 No row, semicircular or parabolic postcloacal crescent of bristles present anterior of cloacal opening 2

1' Semicircular continuous row of bristles anterior of cloacal opening, angled postcloacal crescent (Fig. 10.2 C) ..... *Gordius difficilis* Smith, 1994

[USA: Massachussetts, Nebraska, North Carolina, Wisconsin]

2(1) Cuticle smooth ..... *Gordius robustus* Leidy, 1851

[Canada: Alberta, probably New Brunswick. USA. Mexico.]

2' Cuticle with polygonal areoles (Fig. 10.3 E) ..... *Gordius attoni* Redlich, 1980

[USA: New York, Canada: Saskatchewan]

Gordiida: Male *Gordionus:* Species

1 Precloacal bristle rows present 2

1' Precloacal bristle rows absent 6

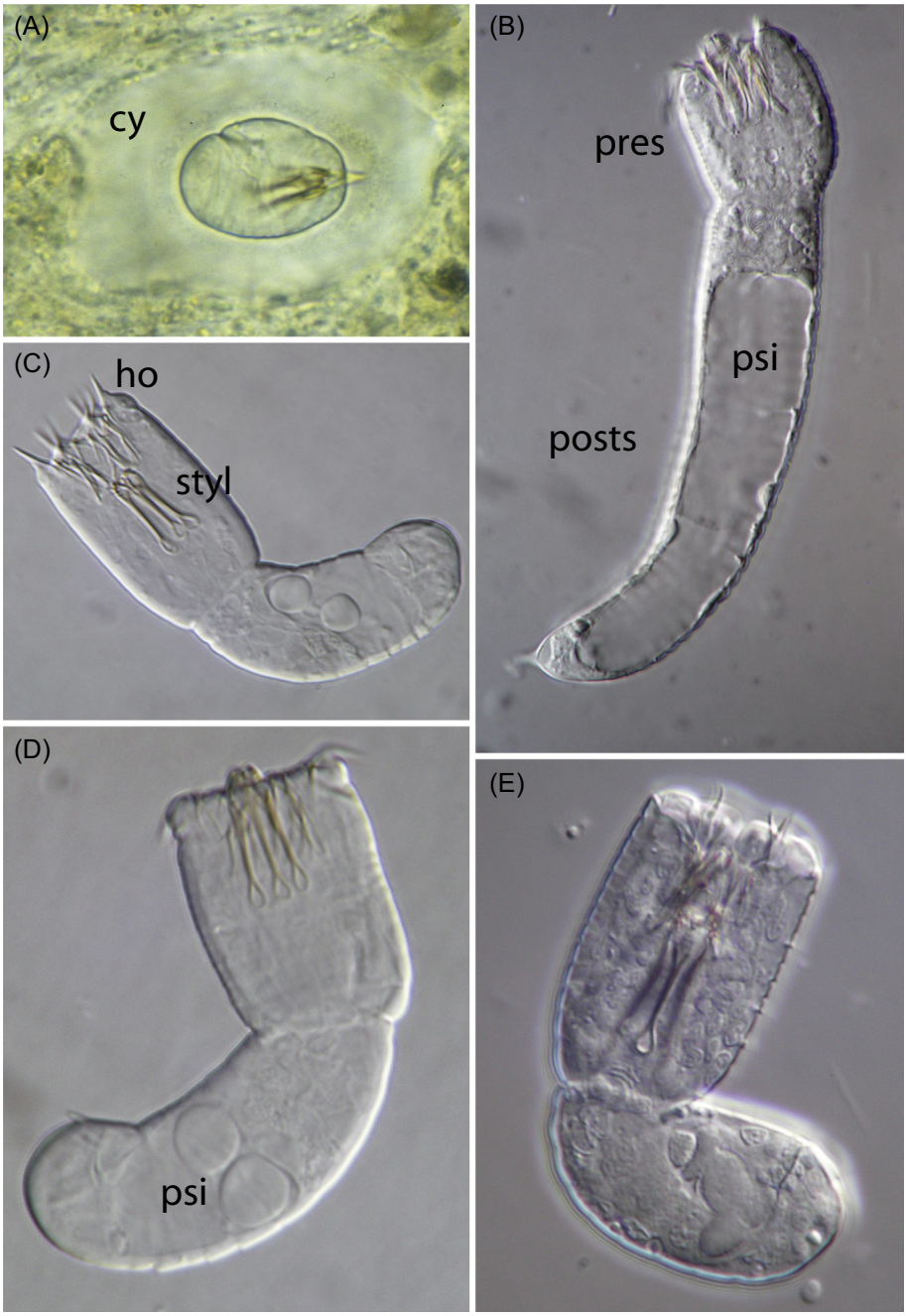

FIGURE 10.5 Larvae. (A) Larva of *Paragordius varius* coiled inside cyst (cy). (B) Larva of *Gordius* sp., entire length about 100 µm. Body is divided into preseptum (pres) and postseptum (posts); latter includes the pseudointestine (psi). (C) Larva of *P. varius* with partly everted hooks (ho) and stylet (styl). (D) Larva of *P. varius* with spherical granules in pseudointestine (psi). (E) Larva of *Neochordodes occidentalis*. Larval length in 3–5 approximately 50 µm.

2(1)	Circumcloacal spines slender and pointed	3
2'	Cloacal opening surrounded by broad, stout spines	*Gordionus lokaaus* Begay, Schmidt-Rhaesa, Bolek & Hanelt, 2012 [USA: New Mexico]
3(2)	Areolar surface smooth	4
3'	"Warty" surface on areoles	*Gordionus platycephalus* (Montgomery, 1898) [USA: Montana, Wyoming, Canada: Quebec]
4(3)	Interareolar spaces narrow, with no or few interareolar structures	5
4'	Interareolar spaces broad, with interareolar structures (Fig. 10.3 G)	*Gordionus violaceus* (Baird, 1853) [USA: Arizona, California, Massachusetts, Missouri, Nebraska]

5(4) Interareolar spaces completely surround areoles (Fig. 10.3 E) ..... *Gordionus lineatus* (Leidy, 1851)
[USA: Maryland, Massachusetts, Michigan, New York, Pennsylvania, Tennessee]

5’ Areoles partly fused with each other ..... *Gordionus bilaus* Begay, Schmidt-Rhaesa, Bolek & Hanelt, 2012
[USA: New Mexico]

6(1) No precloacal ridge 7

6’ Precloacal ridge present ..... *Gordionus alascensis* (Montgomery, 1907)
[USA: Alaska]

7(6) Polygonal areoles, interareolar structures present, no precloacal bristles (Fig. 10.2 H)..... *Gordionus sinepilosus* Schmidt-Rhaesa, Hanelt & Reeves, 2003
[Canada: British Columbia]

7’ Elongate areoles, interareolar structures absent ..... *Gordionus longareolatus* (Montgomery, 1898)
[USA: California]

Gordiida: Male *Neochordodes:* Species

1 Cuticle areoles equally distributed 2

1’ Areoles may be clustered, with broad spaces between ..... *Neochordodes nietoi* Caballero, 1936
[“Mexico.” The cuticular structure of *N. nietoi* needs confirmation by SEM]

2(1) Areoles smooth (Fig. 10.3 A) ..... *Neochordodes occidentalis* (Montgomery, 1898)
[USA: California, Montana, Nebraska, Nevada, Texas, Utah, Washington, Wyoming. Mexico: San Francisco, Veracruz]

2’ Areoles with small marginal knobs ..... *Neochordodes californensis* De Miralles & De Villalobos, 1995
[USA: California]

Gordiida: Male *Pseudochordodes:* Species

1 Both areole types smooth, second type enlarged, darker and bulging 2

1’ First type of areoles smooth, second type with “warty” surface ..... *Pseudochordodes gordioides* (Montgomery, 1898)
[USA: Arizona, California, Montana, Utah]

2(1) Larger areole clusters (of 3 or more) abundant 3

2’ Majority of larger areoles in clusters of two, single ones and few larger clusters can occur (Fig. 10.3 C)..... *Pseudochordodes manteri* Carvalho, 1942
[USA: Nebraska, Mexico: Veracruz]

3(2) Areoles in large clusters 4

3’ Many single large areoles and moderate number of larger clusters ..... *Pseudochordodes meridionalis* Carvalho & Feio, 1950
[Mexico: Tamanlipas]

4(3) Most clusters contain 4 areoles ..... *Pseudochordodes texanus* Schmidt-Rhaesa, Hanelt & Reeves, 2003
[USA: Texas]

4’ Most clusters contain more than 4 areoles (Fig. 10.3 D)..... *Pseudochordodes bulbareolatus* Schmidt-Rhaesa & Menzel, 2005
[Mexico: Veracruz]

Gordiida: Female: Species

Apart from the three species *Gordius robustus*, *Paragordius varius*, and *Chordodes morgani* that can be determined directly with keys above, female diagnosis is quite difficult. Please note that even species from different genera cannot be distinguished with certainty in females. The following key therefore has to be used with great caution.

1 Areoles of two types 2

1’ All areoles of one type 7

2(1) The two types of areoles differ in size, both have a smooth surface 3

2’ Both areole types with different surfaces: first type of areoles smooth, second type with “warty” surface..... *Pseudochordodes gordioides* (Montgomery, 1898)
[USA: Arizona, California, Montana, Utah]

3(2) Larger areoles are separated from each 4

3' Larger areoles are partly fused with each other ..... *Parachordodes tegonotus* Poinar, Rykken & LaBonte, 2004 [USA: Oregon]

4(3) Larger clusters (of 3 and more areoles) are more abundant 5

4' Majority of larger areoles in clusters of two, single ones and few larger clusters can occur (Fig. 10.3 C) ..... *Pseudochordodes manteri* Carvalho, 1942 [USA: Nebraska, Mexico: Veracruz]

5(4) Larger clusters of areoles 6

5' Many single large areoles and moderate number of larger clusters ..... *Pseudochordodes meridionalis* Carvalho & Feio, 1950 [Mexico: Tamanlipas]

6(5) Most clusters contain 4 areoles ..... *Pseudochordodes texanus* Schmidt-Rhaesa, Hanelt & Reeves, 2003 [USA: Texas]

6' Most clusters contain more than 4 areoles (Fig. 10.3 D) ..... *Pseudochordodes bulbareolatus* Schmidt-Rhaesa & Menzel, 2005 [Mexico: Veracruz]

7(1) Areoles cover the entire body, with no or only small grooves between them 8

7' Areoles may be clustered, with broad spaces to other areoles ..... *Neochordodes nietoi* Caballero, 1936 ["Mexico." The cuticular structure of *N. nietoi* needs confirmation by SEM]

8(7) Areole surface smooth 9

8' Areoles with small marginal knobs ..... *Neochordodes californensis* De Miralles & De Villalobos, 1995 [USA: California]

9(8) Areoles separated from each other 10

9' Areoles partly fuse with each other ..... *Gordionus bilaus* Begay, Schmidt-Rhaesa, Bolek & Hanelt, 2012 [USA: New Mexico]

10(9) Interareolar structures present ..... *Gordionus longareolatus, G. platycephalus, G. sinepilosus* or *G. violaceus*

10' Interareolar structures absent ..... *Gordius difficilis, G. attoni, Gordionus alascensis, G. lineatus, G. lokaaus* or *Neochordodes occidantalis*

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