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Organisation of the musculature of *Batillipes pennaki* (Arthrotardigrada, Tardigrada)

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Abstract

The organisation of the musculature of *Batillipes pennaki* Marcus, 1946 is examined by the staining of f-actin with TRITC conjugated phalloidin in conjunction with confocal laser scanning microscopy. The somatic musculature is cross-striated and the muscular architecture can be divided into dorsal, ventral, lateral, ventrolateral, dorsoventral and muscles of the legs. The dorsal longitudinal musculature is formed by two rows of consecutively arranged muscles. Two muscles interconnect these two rows. In comparison the ventral longitudinal musculature is much more complex. The legs are supplied with muscles originating from dorsal, ventral and lateral. In contrast to previously investigated eutardigrades it is noticeable that *B. pennaki* possesses much fewer dorsal attachment points. A similarity is the highly heterogeneous number of the leg muscles of the different legs within one species. Except of the arrangement of the legs and the leg muscles no kind of seriality could be detected.

Keywords: phalloidin, f-actin, musculature, Heterotardigrada

Introduction

Tardigrades are micrometazoans, which are separated into two major probably monophyletic taxa, the Heterotardigrada and Eutardigrada (Jørgensen & Kristensen 2004, Nichols et al. 2006, Sands et al. 2008, Jørgensen et al. 2010). A third taxon, the Mesotardigrada, has been reported by Rahm (1937), but was never found again since then because the collection site was destroyed (Kinchin 1994). Tardigrada play an important role in the discussion of phylogenetic relationships of Arthropoda, namely the Articulata- and the Ecdysozoa-concept (Schmidt-Rhaesa et al. 1998). Tardigrades represent character states that under the light of the different hypotheses either can be interpreted as primary or as secondary (Schmidt-Rhaesa 2001). In the light of the Articulata-hypothesis it is probable that miniaturization and loss of, e. g., coelomic cavities is a secondary feature. In the light of the Ecdysozoa-hypothesis, where tardigrades are assumed to be basal within the Arthropoda, miniaturization could be explained as primary feature. For this discussion it is important to understand the amount to which Tardigrada show segmental patterns. Therefore it is interesting to know the architecture of the inner structures, in particular of the musculature, which shows a highly segmental pattern in arthropods. The study of the musculature of tardigrades has

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a long history, beginning with light microscopical investigations in the nineteenth century (e. g. Plate 1889, Basse 1906, Marcus 1929) and being continued with transmission electron microscopical investigations in the twentieth century (Walz 1973, 1974; Shaw 1974; Greven & Grohé 1975; Kristensen 1978). In the last few years these results were complemented by histochemical staining of f-actin in conjunction with confocal laser scanning microscopy (Schmidt-Rhaesa & Kulessa 2007, Halberg et al. 2009). Marcus (1929) already summarized the descriptions of the musculature of *Echiniscoides sigismundi*, *Echiniscus parvulus*, *Batillipes mirus* and *Tetrakentron synaptae*, nevertheless, most investigations concentrate on eutardigrades and the taxon Heterotardigrada is underrepresented and therefore *Batillipes pennaki* (Fig. 1A,B) was chosen.

Material and methods

In September 2010 specimens of *Batillipes pennaki* were collected from sediment directly in front of the Marine Biological Station in Roscoff, France. The sediment was treated with 7 % $MgCl_2$ and samples were sieved with a net (mesh size 35 µm).

SEM-preparation. Specimens of *B. pennaki* were relaxed with 7 % $MgCl_2$, fixed in 2.5 % glutaraldehyd, dehydrated, critically point dried and sputter-coated with carbonite. Observation took place under a LEO 1525 (Zeiss).

Immunohistochemistry. Specimens of *B. pennaki* were either relaxed with 7 % $MgCl_2$ or left unrelaxed. Before fixation specimens were treated with liquid N_2 and 45 s ultrasonication. Specimens were fixed in 4 % paraformaldehyd in 0.1 M phosphate-buffered saline (PBS, pH 7.4) overnight on ice and fixation was stopped by washing with PBS for 3 × 20 min. Samples were pre-incubated with pre-incubation buffer (PIB, 0.5 % bovine serum albumin, 6 % normal goat serum and 2 or 4 % Triton X-100) overnight on ice. Samples were incubated with 4 or 8 µl of a 25.5 µmol/l basis solution of phalloidin-TRITC (Sigma) in 200 µl PIB overnight on ice and stained animals were embedded in Citifluor on microscopial slides. Samples were investigated with a confocal laser scanning microscope Leica TCS-SPE. ImageJ software (ImageJ 1.43m, National Institutes of Health, USA) was used to post-process and colour code (by depth) the data.

The success rate of the phalloidin-staining was about 18 %. 19 individuals could be used for this investigation. Just one animal could be used for the analysis of the dorsal attachment points and the dorsal longitudinal musculature.

Results

The nomenclature for tardigrade musculature is heterogeneous. The nomenclature used here follows the one used by Schmidt-Rhaesa and Kulessa (2007) for eutardigrades (see table 1 in Halberg et al. 2009 for a comparison of nomenclature), without any intention of homologising these muscles between Heterotardigrada and Eutardigrada.

The signal from histochemical-staining of f-actin does not allow the recognition of cell boundaries, therefore it cannot be decided whether the signal represents single muscle cells or bundles of muscle cells. In the following the term "muscle" will be used for any kind of muscular structure distinctly separated from other such structures, without referring to any kind of organisation.

The somatic musculature of the investigated species can be grouped into dorsal, ventral, lateral, dorsoventral and the leg musculature. Additionally there are attachment points at which the musculature attaches. Due to the bilateral organisation of tardigrades all descriptions only account for one body side. A schematic overview of the musculature is given in figures 5 and 6.

Dorsal attachment points and dorsal longitudinal musculature

Attachment points are labelled alphabetically in large letters from anterior to posterior.

There are thirteen dorsal attachment points (A'–L') (Fig. 2A). All dorsal attachment points are positioned dorsolaterally. The main attachment points in the anterior (A–A') and posterior (L–L') part of the animal are double points, with A' and L' each being the inner attachment points. The resolution in the z-axis is relatively low, therefore it cannot be decided for every attachment point with certainty whether the dorsal longitudinal musculature attaches or not. Attachment points, which are associated with the dorsal longitudinal musculature are A, A', C, E, L and L'. For attachment point G it is hard to decide whether parts

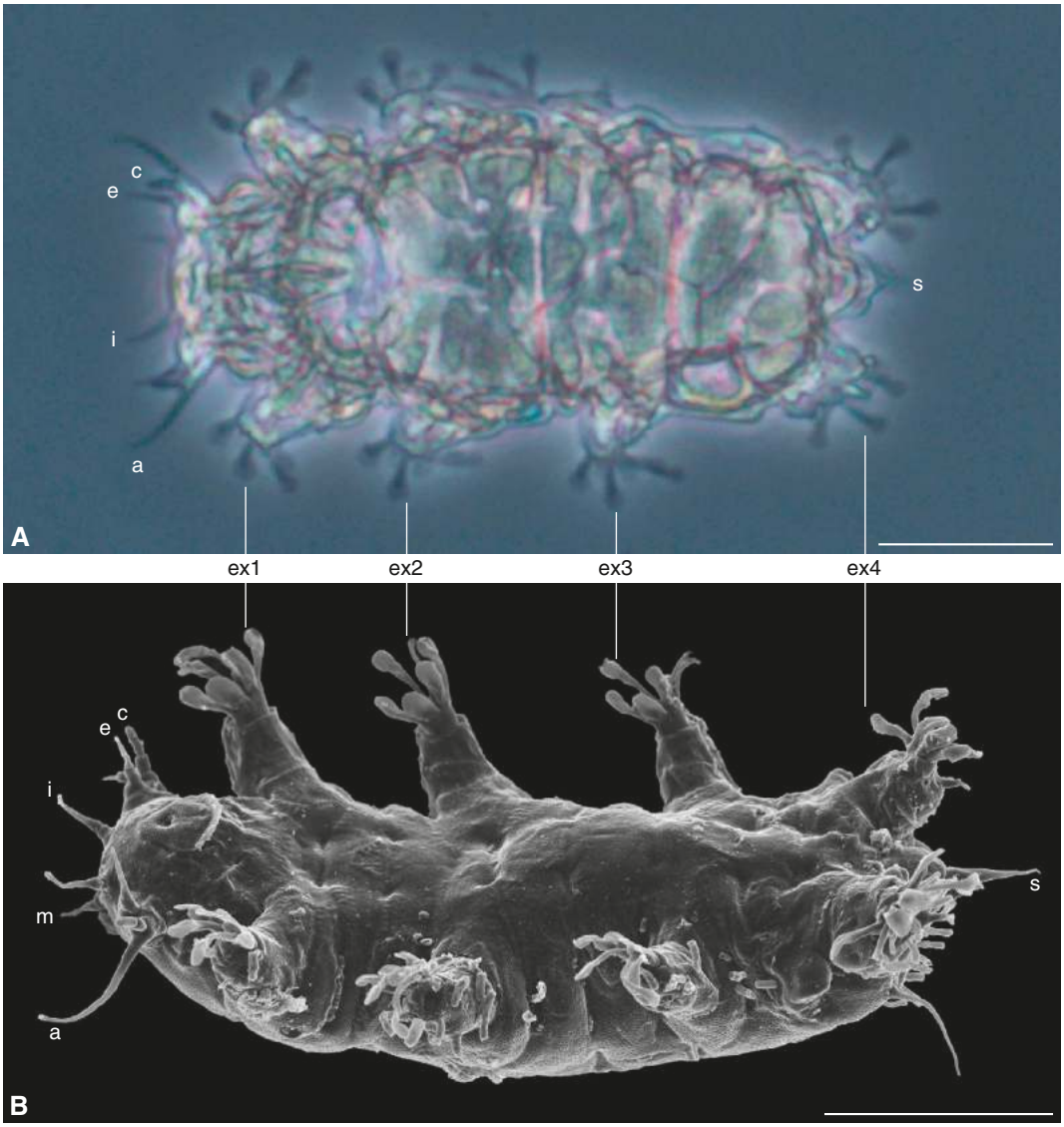

Fig. 1. *Batillipes pennaki*. **A.** dorsal overview (light micrograph). **B.** lateral overview (SEM micrograph). Abbreviations: **a**, lateral cirri A (paired); **c**, clavae (paired); **e**, external medial cirri (paired); **ex1,** first leg; **ex2**, second leg; **ex3**, third leg; **ex4**, fourth leg; **i,** internal medial cirri (paired); **m**, median cirrus (unpaired); **s,** caudal spine. Scale bars 25 µm.

of the dorsal longitudinal musculature attach or not. It seems as if four muscles come together in H, therefore this also is interpreted as attachment point. To attachment points B, C, D, E, F, G, I and K dorsal leg muscles are attached (Fig. 2A).

The dorsal longitudinal musculature of *Batillipes pennaki* consists of dorsolateral muscles that originate anteriorly at attachment points A and A' and terminate posteriorly at attachment points L and L'. Two well distinguishable muscles originate at each of the attachment points A and A' whereas only one muscle terminates at attachment point L and two at L' (Fig. 2A).

The outermost muscle originates at attachment point A and takes course to attachment point H. The second muscle that originates at A runs to

attachment point E, as well as the outer of the muscles that originates at attachment point A'. The innermost muscle originates at A' and attaches at attachment points C and E. From E two muscles run posteriorly. The innermost muscle runs to attachment point G, although the course of this muscle does not change, the muscle might attach at G. From G this muscle runs straight on and terminates at attachment point L'. The second muscle that attaches at E lies in the middle and takes course to attachment point H. From H two muscles run posteriorly. The median of these muscles terminates at attachment point L' and the outer of these muscles at attachment point L (Fig. 2A).

Ventral attachment points and ventral longitudinal musculature. Attachment points are labelled alphabetically in small letters from anterior to posterior.

There are six ventral attachment points (a'–e) (Fig. 2B). They are positioned ventrolaterally and are, probably except of d, associated with the ventral longitudinal musculature. In the anterior part of the animal two attachment points exist, from which ventral longitudinal muscles originate. These are attachment points a' and a. Both are located anterior of the pharyngeal bulb. Attachment point a' is positioned in the anteriormost region of the animal, whereas a is located slightly more posteriomedially. In the posterior body region only one attachment point (e) is found, at which the ventral longitudinal musculature terminates (Fig. 2B).

The ventral musculature is composed of two muscles that run in parallel. The muscles alternatively originate at attachment point a' or a and both terminate at attachment point e. The outer muscle that originates at a' runs to attachment point b, lateral of the pharyngeal bulb. Posterior of b it attaches at attachment point c and terminates at e. The inner muscle originates at a and again attaches at c. It then ends at attachment point e. Attachment point d seems not to be associated with one of these muscles. Apparently it is only associated to muscles originating from the uneven ventromedian attachment points 3 and 5. Posterior of attachment point e one muscle (eiii) runs directly into the fourth leg (Fig. 2B).

Ventromedian attachment points. On the ventral body-side there are seven median attachment points (1–7) (Figs. 2B, 3). The even as well as the uneven median attachment points 1 and 3 are directly positioned in the midline. In contrast, median attachment points 5 and 7 seem to be clearly separated and lie lateral of the midline. From the median attachment points muscles run to the legs, to the lateral musculature and dorsoventrally (Figs. 2B, 3).

Muscles that are associated with the median attachment points are named after their originating median attachment point. Muscles that run directly lateral into a leg as well as muscles which process into an anterior positioned leg are labelled i. They are numbered by their relative position in which numbering starts with the muscle in the nearest position to the ventral midline and proceeds in clockwise direction. If corresponding muscles are missing, numbering may not start with 1. Muscles running into a posterior positioned leg are labelled iii. Such muscles are only present at median attachment point 7 and run into the fourth leg, which is backward orientated. Because of this different position numbering starts with the muscle positioned nearest to the midline and then proceeds counterclockwise. Muscles directed to the dorsal part of the animal are labelled ii. Muscles which are interconnected to or are a part of the ventral or lateral musculature are labelled a, depending on the direction of their alignment they are additionally labelled with $_a$ for anterior or $_p$ for posterior.

From every even median attachment point just one muscle originates. The number of originating muscles from uneven median attachment points differs.

Lateral longitudinal, ventrolateral and dorsoventral musculature. The course of the lateral musculature could only be observed in parts and in very few animals. Lateral attachment points are labelled $l_{(a–i)}$ from anterior to posterior (Fig. 3).

The lateral attachment points l_c and l_f are only associated to leg muscles, whereas the others are associated to leg and dorsoventral muscles. The dorsoventral musculature is labelled ii. Not all dorsoventral muscles originate from ventromedian attachment points, some of these muscles originate from ventral attachment points. This is indicated by the number or small letter of the corresponding originating ventromedian or ventral attachment point, respectively.

The lateral musculature seems to be formed by two main muscles (l1 and l2) running in parallel from anterior to posterior, although the exact

course could not be followed (Fig. 4). Two muscles from the ventral part of the animal are associated to the lateral musculature. One of these muscles (ea) originates from ventral attachment point e and one (7a) from ventromedian attachment point 7 (Figs. 3, 4). Other recognisable muscles that are associated to the lateral musculature are muscles that are also associated to the legs. They are labelled $l_{(a\text{-}i)}$ and, depending to which leg they are associated, they are labelled 1, 2 or 3 for the first, the second or the third leg, respectively. Two muscles each are running into the legs 1-3. These are l_b-1 and l_c-1 for the first, l_e-2 and l_f-2 for the second and l_h-3 and l_i-3 for the third leg (Figs. 3, 4).

From the uneven ventromedian attachment points 1, 3, 5 and 7 muscles run to ventral attachment points, one muscle each from ventromedian attachment points 1, 5 and 7 and two from ventromedian attachment point 3 (Fig. 2B). From ventromedian attachment point 1 one muscle ($1a_p$) in posterior direction is connected to ventral attachment point c. From ventromedian attachment point 3 two muscles originate. One ($3a_a$) runs to the more anterior positioned ventral attachment point c and one ($3a_p$) to the more posterior positioned d. Muscle $5a_a$ from ventromedian attachment point 5 directs to the anterior and terminates at ventral attachment point d. Muscle $7a_a$ originates from ventromedian attachment point 7 and terminates at the more anterior positioned ventral attachment point e (Fig. 2B).

B. pennaki possesses seven dorsoventral muscles. They originate alternately from a ventromedian or a ventral attachment point (Fig. 3). The first (1ii), the third (3ii), the fifth (5ii) and the seventh (7ii) dorsoventral muscle originate from ventromedian attachment points 1, 3, 5 and 7, respectively. The second (cii), the fourth (dii) and the sixth (eii) dorsoventral muscle originate from the ventral attachment points c, d and e, respectively. From the ventral side they process to the dorsal side of the animal. None of the muscles terminates at a dorsal attachment point or at the dorsal longitudinal musculature, instead they terminate at attachment points associated to the lateral musculature. The dorsoventral muscle 1ii terminates at lateral attachment point l_a, cii at l_b, 3ii at l_d, dii at l_e, 5ii at l_g, eii at l_h and 7ii at l_i (Fig. 3).

Leg musculature. Each leg is equipped with dorsal, ventral and in part with lateral muscles (Figs. 2A, B, 3, 4). Legs 2 and 3 have the same set of ventral and lateral muscles, but may differ in the set of the dorsal leg muscles. The first leg differs in the number of dorsal and ventral muscles from the second and third leg. The fourth leg is backward orientated and its muscle set differs remarkably from the ones of the first three legs. The first leg possesses 14, the second 10, the third 9 and the fourth 6 leg muscles.

Dorsal. The muscles are labelled with small letters referring to the dorsal attachment point they are originating from and a number indicating the quantity of dorsal leg muscles originating from the dorsal attachment point. Even though there is always only one muscle originating from one attachment point, numbers are used to prevent confusion with the naming of the ventral attachment points. The anteriomost dorsal leg muscle of the legs 1–3 is a very short muscle originating and terminating in the middle of each leg. Attachment points for these muscles are labelled X, Y and Z for leg 1, 2 and 3, respectively. Legs 1 and 2 also possess a posterior muscle originating from attachment points T1 and T2, respectively. For leg 3 such a muscle could not be detected with certainty.

In the first leg six dorsal muscles exist (Fig. 2A). The anteriomost muscle (x1) is very short and originates at about half of the leg at attachment point X. It terminates shortly behind its origin. Four muscles (b1, c1, d1 and e1) originate from the intermediate attachment points B, C, D and E. The sixth muscle (t1) originates near muscle d1 at attachment point T1 (Fig. 2A).

The second leg possesses four dorsal leg muscles (Fig. 2A). One (f1) originates from intermediate attachment point F, one (g1) from intermediate attachment point G. The other two muscles (y1 and t2) originate from different attachment points. These are attachment points Y for the more anterior positioned (y1) and T2 for the more posterior positioned (t2) muscle (Fig. 2A).

The muscle set for the third leg is similar to the muscle set of the second leg (Fig. 2A). Two muscles (i1 and k1) originate from intermediate attachment points I and K, and one (z1) from a different attachment point (Z). A more posterior positioned muscle like t2 could not be detected with certainty (Fig. 2A).

In the fourth leg no muscle originating from

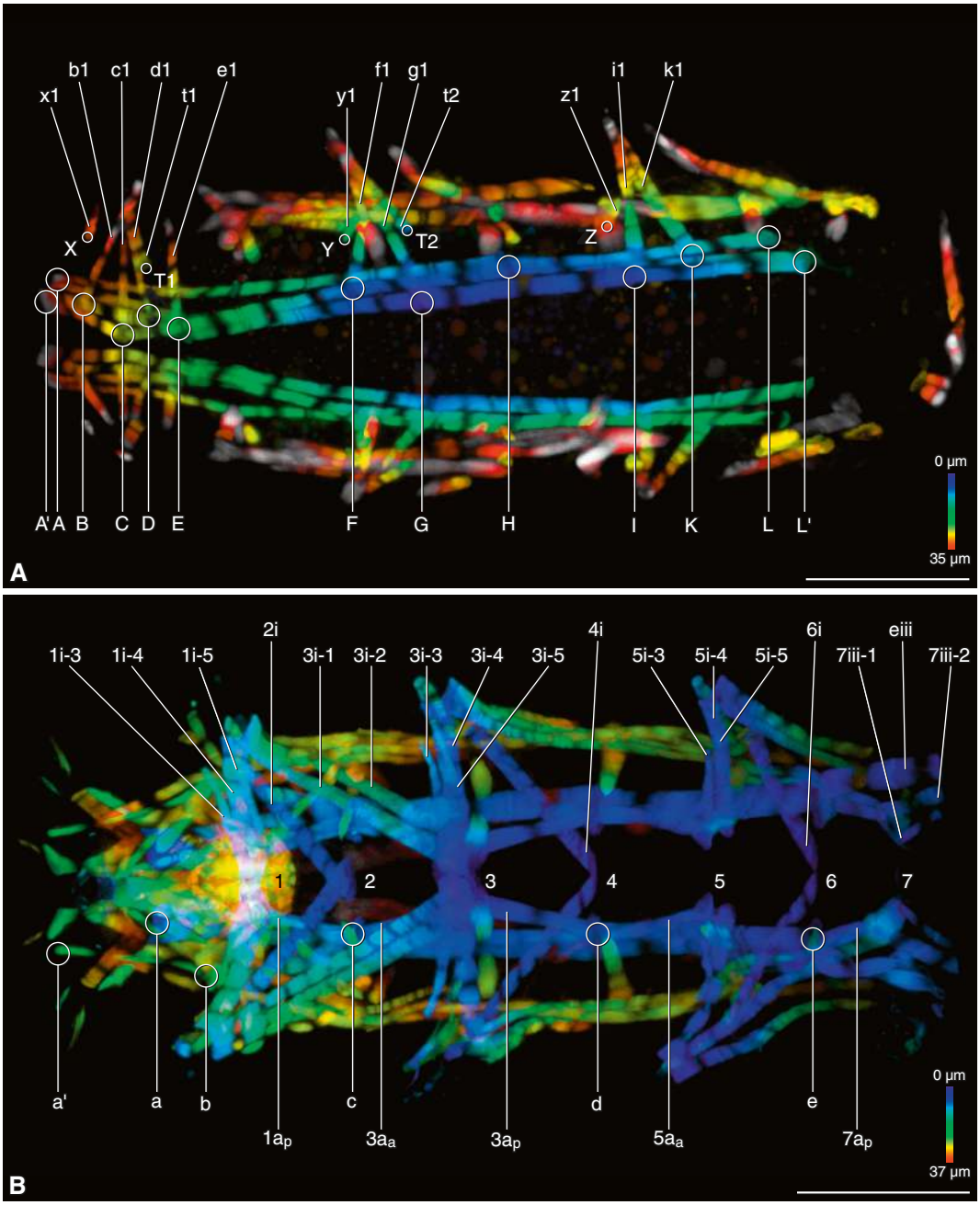

Fig. 2. Visualization of the dorsal and ventral musculature of *Batillipes pennaki* by f-actin staining and cLSM. Maximum intensity projections colour-coded by depth. **A.** Overview of the dorsal attachment points, the dorsal musculature and the dorsal leg muscles. Dorsal attachment points A'–L'. Attachment points of the legs **1.** X, T1; **2.** Y, T2; **3.** Z. Dorsal leg muscles **1.** x1, b1, c1, d1, t1, e1; **2.** y1, f1, g1, t2; **3.** z1, i1, k1, t3. **B.** Overview of the ventromedian and ventral attachment points, the ventral and ventrolateral musculature and the ventral leg muscles. Ventromedian attachment points 1–7. Ventral attachment points a'–e. Ventrolateral muscles $1a_p$, $3a_a$, $3a_p$, $5a_a$, $7a_p$. Ventral leg muscles **1.** 1i-3, 1i-4, 1i-5, 2i, 3i-1, 3i-2; **2.** 3i-3, 3i-4, 3i-5, 4i; **3.** 5i-3, 5i-4, 5i-5, 6; **4.** eiii, 7iii-1, 7iii-2. Scale bars 25 µm.

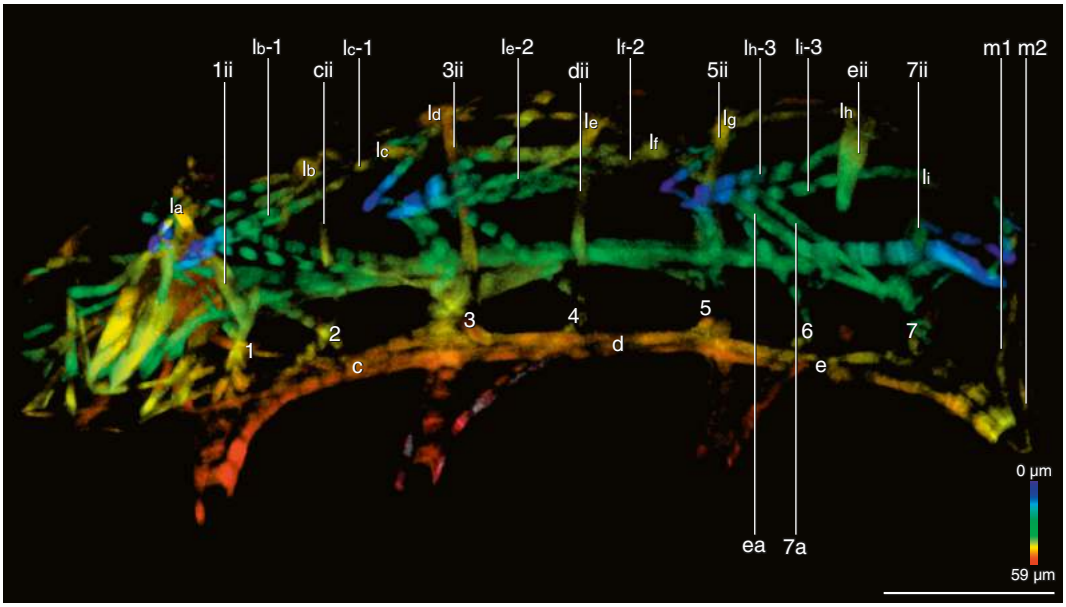

Fig. 3. Visualization of the lateral musculature of *Batillipes pennaki* by f-actin staining and cLSM. Maximum intensity projections colour-coded by depth. **A.** Overview of the lateral attachment points, the dorsoventral and ventrolateral muscles and the lateral leg muscles. Lateral attachment points l_a–l_i. Dorsoventral muscles 1ii, cii, 3ii, dii, 5ii, eii, 7ii. Ventrolateral muscles ea, 7a. Lateral leg muscles **1.** l_b-1, l_c-1; **2.** l_e-2, l_f-2; **3.** l_h-3, l_i-3. Dorsal leg muscles **4.** m1, m2. Ventromedian attachment points 1–7. Ventral attachment points c–e. Scale bar 25 µm.

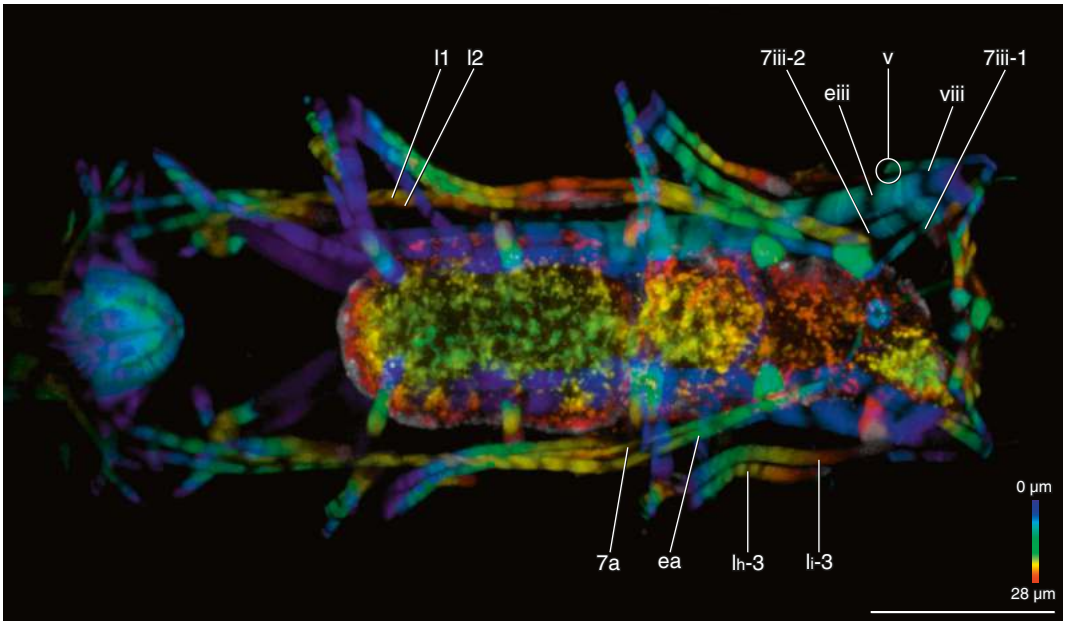

Fig. 4. Visualization of parts of the ventral, lateral and leg musculature of *Batillipes pennaki* by f-actin staining and cLSM. Maximum intensity projections colour-coded by depth. **A.** Lateral muscles l1, l2. Leg muscles **3.** l_h-3, l_i-3; **4.** eiii, 7iii-1, 7iii-2, viii with attachment point v. Scale bar 25 µm.

a dorsal attachment point could be detected with certainty. Nevertheless, at least two dorsal leg muscles (m1 and m2) run into the fourth leg (Fig. 3). No attachment points can be assigned to them, but due to their dorsal origin these muscles are labelled here m as "regular" dorsal leg muscles.

Ventral. The ventral leg musculature, except of two muscles, is only associated to the ventromedian attachment points. One of these exceptional muscles originates from ventral attachment point e and therefore in a sense constitutes a projection of the ventral longitudinal musculature and the other muscle (viii) originates from a different attachment point (v, see below) (Figs. 2B, 4). Ventral leg muscles that are aligned to the anterior of the animal are labelled i, ventral leg muscles that are aligned to the posterior of the animal are labelled iii.

Most ventral leg muscles are terminating at the tip of the legs, except of the anteriomost ventral muscle (i-3). This muscle terminates at about half the length of the legs.

The first leg possesses six ventral muscles (Fig. 2B). The most anterior leg muscle (1i-3) originates from median attachment point 1. From median attachment point 1 two further muscles (1i-4 and 1i-5) run into the first leg. One muscle (2i) originates from the second median attachment point and two muscles (3i-1 and 3i-2) from the third attachment point (Fig. 2B).

The second and third leg possess four ventral muscles each (Fig. 2B). These are the muscles 3i-3, 3i-4, 3i-5 and 4i for the second and 5i-3, 5i-4, 5i-5 and 6i for the third leg (Fig. 2B).

The fourth leg receives four ventral leg muscles. Two (7iii-1 and 7iii-2) are originating from median attachment point 7 and one (eiii) originates from ventral attachment point e (Figs. 2B, 4). The fourth ventral leg muscle (viii) originates from attachment point v from about half the length of the leg and ends at the tip of it (Fig. 4).

Lateral. Each leg, except of the fourth, has two lateral muscles (Figs. 3, 4). Muscles are labelled $l_{(a-i)}$ referring to the lateral attachment point they are originating from and numbered 1, 2 or 3 depending to which leg they are associated.

The first leg possesses lateral muscles l_b-1 and l_c-1, the second l_e-2 and l_f-2 and the third l_h-3 and l_i-3 originating from lateral attachment points l_b, l_c, l_e, l_f, l_h and l_i, respectively (Figs. 3, 4).

Discussion

Problems in the preparation of *Batillipes pennaki*. The staining of the musculature of *B. pennaki* turned out to be difficult, just about 18 % of the individuals used showed some kind of signal. Within these 18% the success of staining still was very heterogeneous. One problem was a partially destroyed musculature, which was probably caused by the treatment with N_2 and ultrasonication. Another problem was that the whole musculature (meaning dorsal and ventral) could never be detected at once. There were, however, no problems with the relaxation of the specimens. Whether they were relaxed with $MgCl_2$ or not made no difference, all specimens can be described as relaxed. Relaxation seems to be a problem only for terrestrial species (see Schmidt-Rhaesa & Kulessa 2007).

A consequence of the high loss rate in the staining procedure was that in spite of the high number of individuals used very few could be used for the analysis of the musculature. Therefore just few replicates were available. Because of this problem the course of not every muscle could be followed as exactly as wished. This accounts especially to the dorsal leg musculature and the lateral musculature. Furthermore, the reliable recognition of attachment points of the musculature was not always clear to make. In some positions the musculature appears as one solid strand which runs in one layer, but locally shows elevations out of this layer, which probably represent attachment points. A comparison with ultrastructure (TEM) would be very helpful.

Comparison with known data on the musculature of *Batillipes mirus*. Since the first described observation of tardigrades in 1773 by J. A. E. Goeze several studies on their musculature have been performed. Marcus (1929) already described the musculature of *Batillipes mirus*. Compared to his detailed study based on light-microscopy we could verify several aspects but can also add some more muscles and question some of his observations.

For better comparison see schematic drawings of the description of the musculature of Marcus and this study (Figs. 5, 6). The description of Marcus (1929) of the gross anatomy of *B. mirus* could be verified but differs in some detail. Marcus describes four dorsal longitudinal muscles for each body-side. These are the medially po-

sitioned (α–ν; Greek symbols represent Marcus' terminology), the connecting (π–ς) and the two laterally positioned (α_1–λ and λ_1–ξ) muscles. Most of the dorsal longitudinal muscles Marcus described are running from anterior to posterior without any attachment points. In contrast we could describe a more complicated distribution pattern, beginning with four muscles in parallel in the head and then three in parallel posterior of the first leg, with attachment points at A, A', C, E, H, L, L' and possibly at G. Muscles E–H and H–L' probably correspond to Marcus' connecting muscle π–ς and muscles A–H and H–L to Marcus' α–ν. In contrast, no correspondence to the lateral muscle λ_1–ξ, positioned in the last leg, could be detected. The dorsally described muscle α_1–λ is probably part of the lateral instead of the dorsal musculature because it is positioned near the outer edge of the animal, where we could locate the lateral musculature of *B. pennaki*. Marcus (1929) description of the dorsal leg muscles differs from ours in the way that he de facto describes three dorsal leg muscles for the first and two muscles each for the second and third leg terminating in the leg. In the first and the second leg these muscles possess six and three origins, respectively. Marcus describes that in the first leg muscle 4 branches into three muscles (S_1, S_2 and S_3) and muscle 8 branches into two muscles (β_1 and β_2), whereas in the second leg muscles 4 and 8 are fused. Additionally, he describes one further muscle for each of the two legs which is muscle 10. In contradiction to Marcus' description in case of the first leg we could identify five single muscles. The same holds true for the second leg, where we also could not detect any fused muscles, instead we could identify three single muscles. Muscles b1, c1 and d1 of the first leg possibly correspond to Marcus' muscle 4 with the origins at S_1, S_2 and S_3 and muscles f1 and g1 probably correspond to muscles 4 and 8 in Marcus' (1929).

Marcus' (1929) description of the ventral longitudinal musculature is very similar to ours. He describes one muscle (A–N) with intermediate attachment points at E and H and a further muscle originating from B. We could also detect two origins for the ventral longitudinal musculature, these are attachment points a' and a. Marcus (1929) describes attachment points A and B as origins for the longitudinal musculature, but could not verify the origin of B. Attachment point b is connected by a muscle to the anterior positioned a'. Muscle a'–b possibly corresponds to Marcus' described muscle V–W_1. Probably attachment point W_1 corresponds to the herein described b. Intermediate attachment point H as well as the last attachment point N possibly correspond to the herein described d and e. In contrast to Marcus we could always identify two muscles running in parallel until the most posterior attachment point e.

The descriptions of the ventral leg muscles are very similar in both studies. From ventromedian attachment points 1, 3 and 5 three muscles each run into the directly lateral positioned leg, these are muscles 1i-3–1i-5, 3i-3–3i-5 and 5i-3–5i-5, respectively. To muscles 1i-3 and 5i-3 probably muscles W_1–x and J–b (Marcus 1929) correspond as well as muscles 1i-4, 3i-4 and 5i-4 to muscle 2 (Marcus 1929) in each leg. In the first legs muscles 3i-1 and 3i-2 correspond to muscles 7 and 3–c–E (Marcus 1929), respectively. Muscle 6 of the first leg and muscles 7 from the second and third leg (Marcus 1929) correspond to muscles 2i, 4i and 6i, respectively. In the fourth leg muscle viii possibly corresponds to muscle 1 (Marcus 1929). Muscles $3a_p$ and $5a_a$ find their counterparts in muscles F–H and H–J, respectively, whereas corresponding muscles for $1a_p$, $3a_a$ and $7a_p$ are missing. Particular for the lateral musculature we could add some more information.

In his ultrastructural study on *Batillipes noerrevangi* Kristensen (1978) showed that the musculature of arthrotardigrades is of a "true" cross-striated organisation. This could be confirmed by the investigation of *B. pennaki*. The organisation of the musculature of arthrotardigrades is in contrast to the musculature organisation in eutardigrades, where only the muscles of the stylet, of the stylet support and of the pharyngeal bulb are cross-striated (Halberg et al. 2009). In contrast, the somatic musculature of eutardigrades seems to be of an intermediate organisation between smooth and obliquely striated (Walz 1974).

Comparison with other tardigrades. Previously performed histochemical studies on the musculature of tardigrades dealt only with specimens belonging to the taxon of Eutardigrada (Schmidt-Rhaesa & Kulessa 2007, Halberg et al. 2009), here we add the first data on the musculature of specimens belonging to the taxon of Heterotardigrada. In general the results among different species of Eutardigrada are comparable. *Batillipes pennaki* instead shows some differences to the previously investigated eutardigrade species.

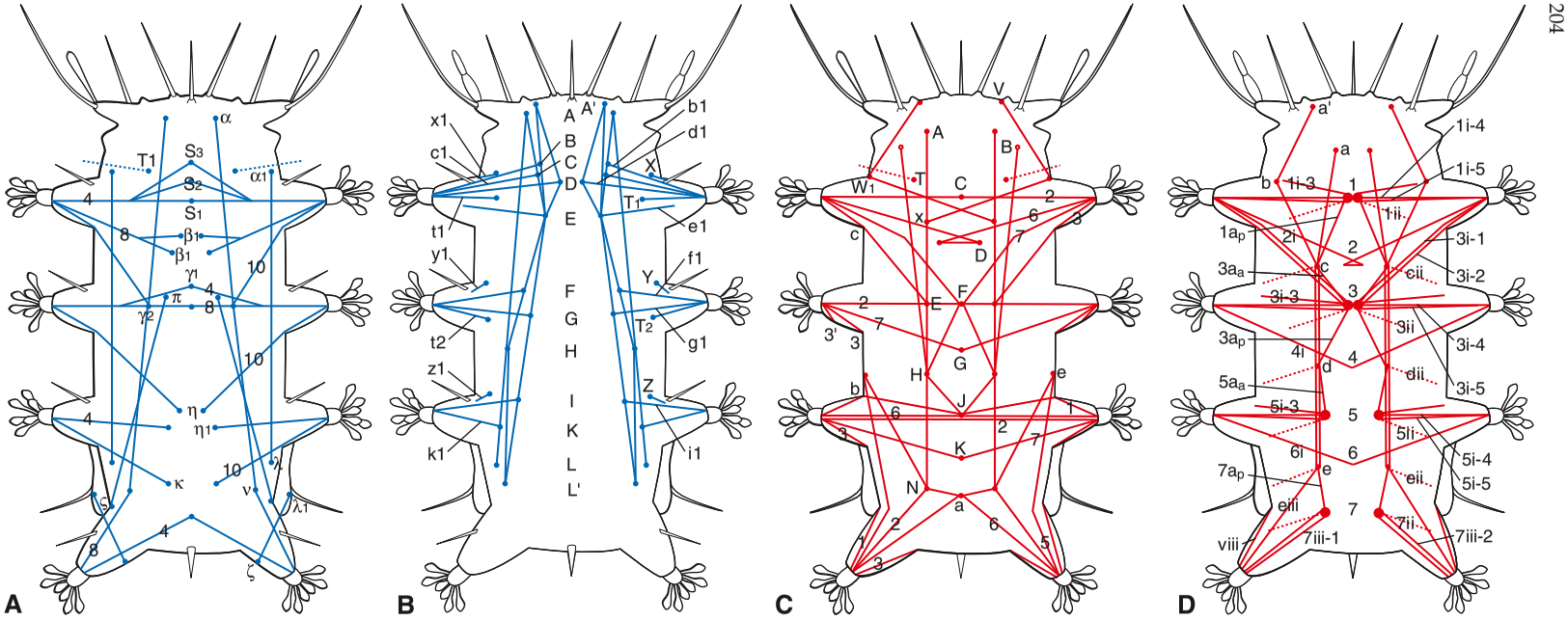

Fig. 5. Schematic drawing of the musculature of *Batillipes*.
A, C. *Batillipes mirus*, redrawn after Marcus (1929). **B, D.** *Batillipes pennaki*, pattern of the f-actin stained musculature in this study.
A, B. Dorsal overview, muscles in blue. **C, D.** Ventral overview, muscles in red.
A. Overview of the dorsal and the leg attachment points and the dorsal longitudinal and leg muscles.
Dorsal attachment points $\alpha, \alpha_1, \pi, \gamma_2, \lambda, \nu, \varsigma, \lambda_1, \xi$. Attachment points of the legs **1.** $S_3, S_2, S_1, \beta_1, \beta_2, \gamma_2$; **2.** γ_1, η; **3.** η_1, κ; **4.** ν.
Dorsal longitudinal muscles α–ν, α_1–λ, π–ς, λ_1–ξ. Dorsal leg muscles **1.** 4, 8, 10; **2.** 4, 8, 10; **3.** 4, 10; **4.** 4, 8.
B. Overview of the dorsal and the leg attachment points and the dorsal longitudinal and leg muscles.
Dorsal attachment points A'–L'. Attachment points of the legs **1.** X, T1; **2.** Y, T2; **3.** Z.
Dorsal leg muscles **1.** x1, b1, c1, d1, t1, e1; **2.** y1, f1, g1, t2; **3.** z1, i1, k1, t3.
C. Overview of the ventromedian and ventral attachment points, the ventral longitudinal, ventrolateral and leg muscles.
Ventromedian attachment points C, D, F, G, J, K, a. Ventral attachment points E, H.
Ventral muscles V–W_1, A–N, B–H. Ventrolateral muscles F–H, H–J. Ventral leg muscles **1.** 2, 3–c–E, 6, 7; **2.** 2, 3, 3', 7; **3.** 1, 2, 3, 6, 7; **4.** 1, 2, 3, 5, 6.
D. Overview of the ventromedian and ventral attachment points, the ventral longitudinal, ventrolateral and leg muscles and the dorsoventral muscles.
Ventromedian attachment points 1–7. Ventral attachment points a', a, b, c, d, e.
Ventrolateral muscles $1a_p, 3a_a, 3a_p, 5a_a, 7a_p$. Ventral leg muscles **1.** 1i-3, 1i-4, 1i-5, 2i, 3i-1, 3i-2; **2.** 3i-3, 3i-4, 3i-5, 4i; **3.** 5i-3, 5i-4, 5i-5, 6; **4.** eiii, 7iii-1, 7iii-2, viii. Dorsoventral muscles 1ii, cii, 3ii, dii, 5ii, eii, 7ii.

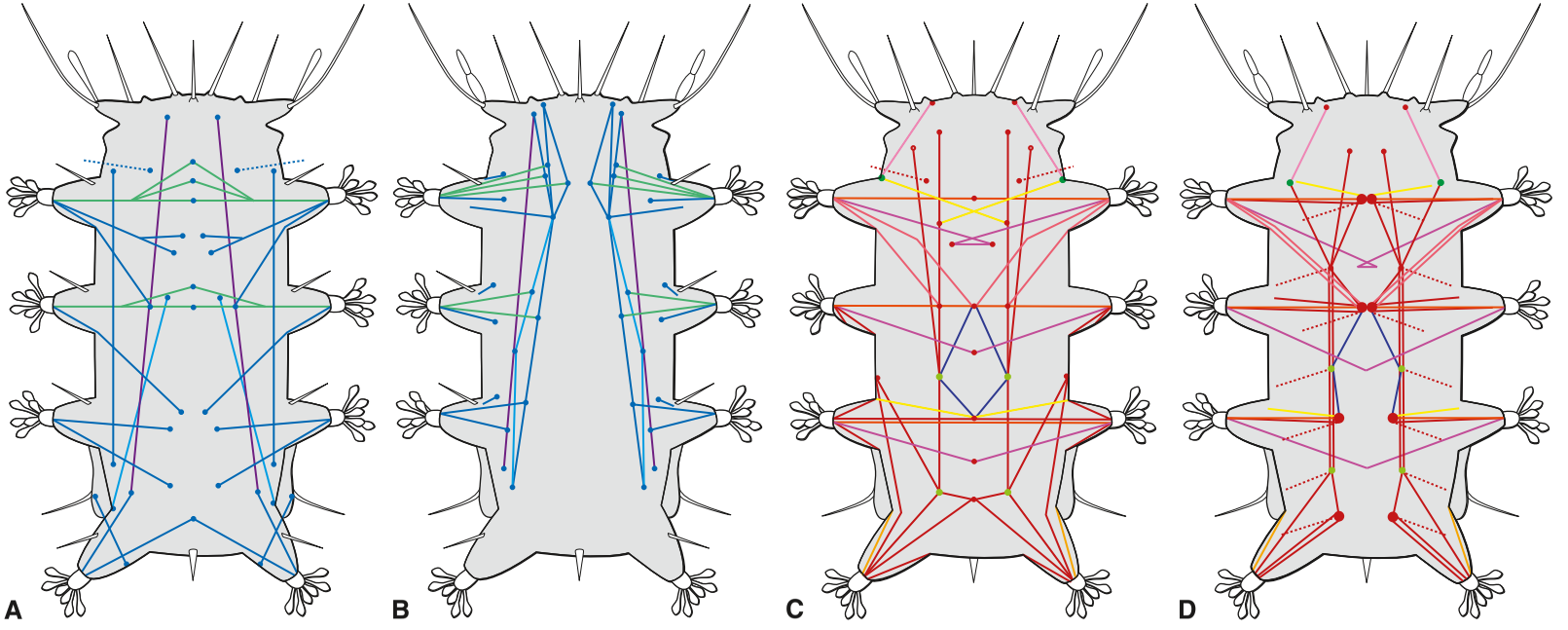

Fig. 6. Schematic drawing of the musculature of *Batillipes*.
A, C. *Batillipes mirus*, redrawn after Marcus (1929). **B, D.** *Batillipes pennaki*, pattern of the f-actin stained musculature in this study.
A, B. Dorsal overview. **C, D.** Ventral overview.
A–D. Ground colour for the dorsal overviews: **dark blue**; for the ventral overviews: **red**. Probably corresponding muscles are coded in the same colour (other than ground colours). For the labelling see figure 5. In the following comparisons first mentioned muscles refer to Marcus, second mentioned to this study.
A, B. Corresponding muscles are probably:
dorsal longitudinal: **violet** α–ν to A–H–L; **light blue** π–ς to E–H–L';
legs: **turquoise** **1.** 4 with origins at S_1–S_3 to b1, c1 and d1; **2.** 4 and 8 to f1 and g1.
C, D. Corresponding muscles are probably:
ventral longitudinal: **rose** V–W_1 to a'–b; **ventrolateral:** **dark violet** F–H and H–J to $3a_p$ and $5a_a$;
legs: **yellow** **1.** W_1–x to 1i-3; **3.** J–b to 5i-3; **orange** **1.** 2 to 1i-4; **2.** 2 to 3i-4; **3.** 2 to 5i-4; **light red** **1.** 7 and 3–c–E to 3i-1 and 3i-2; **pink** **1.** 6 to 2i; **2.** 7 to 4i; **3.** 7 to 6i; **light orange** **4.** 1 to viii.
Corresponding attachment points are probably: **dark green** W_1 to b; **light green** H and N to d and e.

The most striking feature is the high difference in the number of dorsal attachment points. *Batillipes pennaki* only shows 13 such points, whereas the investigated eutardigrades have between 19 and 25 dorsal attachment points. In all tardigrade species investigated so far the leg musculature is a very heterogeneous feature, wherein even the different legs of one species possess different numbers of muscles. The number of leg muscles varies in every leg for every species so that they are not comparable. In all species the fourth leg possesses the fewest muscles, which is probably due to its backward orientation and its different role.

A common feature of the longitudinal musculature of all tardigrades described so far is that these muscles appear to run continuously from anterior to posterior. When they attach to intermediate attachment points, these points do not show a clear segmental position.

The descriptions of the musculature of the heterotardigrades *Echiniscoides sigismundi*, *Echiniscus parvulus* and *Tetrakentron synaptae* of Marcus (1929) resemble each other in the way that they all show a dorsal musculature with just few dorsal attachment points, a differing number of leg muscles and in contrast to the dorsal musculature a relatively complex ventral musculature. We regard a closer comparison among heterotardigrades as premature, because Marcus (1929) could observe only part of the muscles in e. g. *T. synaptae* and because unpublished own results on representatives of the Echiniscoidea indicate some differences to Marcus' descriptions.

Consequences for muscle evolution within Arthropoda (= Panarthropoda). The only segmental pattern in the muscle system of tardigrades is the presence of four legs and the presence of four iterated sets of leg muscles. As these sets do not correspond exactly to each other in the number of muscles and their course, even the leg muscles do not constitute a "real" segmental pattern in the sense of an iterated series of structures. As has already been discussed by Schmidt-Rhaesa & Kulessa (2007), this can be interpreted in two ways. Either the muscular patterns present in tardigrades derive from segmental patterns and were modified during the process of miniaturization or segmentation evolved successively in different organ systems within Arthropoda (= Tardigrada + Onychophora + Euarthropoda = Panarthropoda).

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References

Basse, A. (1906). Beiträge zur Kenntnis des Baues der Tardigraden. Zeitschrift für Wissenschaftliche Zoologie 80: 259–281.

Goeze, J. A. E. (1773). Ueber den kleinen Wasserbär. In: Herrn Karl Bonners Abhandlungen aus der Insektologie, Goeze, J. A. E. (ed.), pp. 367–375. Gebauer, Halle.

Greven, H. & G. Grohé (1975). Die Feinstruktur des Integumentes und der Muskelansatzstellen von *Echiniscoides sigismundi* (Heterotardigrada). Helgoländer wissenschaftliche Meeresuntersuchungen 27: 450–460.

Halberg, K. A., D. Persson, N. Møbjerg, A. Wanninger & R. M. Kristensen (2009). Myoanatomy of the marine tardigrade *Halobiotus crispae* (Eutardigrada: Hypsibiidae). Journal of Morphology 270: 996–1013.

Jørgensen, A. & R. M. Kristensen (2004): Molecular phylogeny of Tardigrada – investigation of the monophyly of Heterotardigrada. Molecular Phylogenetics and Evolution 32: 666–670.

Jørgensen, A., Faurby, S., Hansen, J. G., Møbjerg, N. & Kristensen, R. M. (2010). Molecular phylogeny of Arthrotardigrada (Tardigrada). Molecular Phylogenetics and Evolution 54: 1006–1015.

Kinchin, I. M. (1994). The biology of tardigrades. Portland Press, London.

Kristensen, R. M. (1978). On the structure of *Batillipes noerrevangi* Kristensen 1978. 2. The muscle-attachments and the true cross-striated muscles. Zoologischer Anzeiger 200: 173–184.

Marcus, E. (1929). 10. Muskulatur. In: Dr. H. G. Bronns Klassen und Ordnungen des Tierreichs, 5. Band, IV. Abt. Buch 3, Teil 1, Tardigrada., pp. 49–66. Akademische Verlagsgesellschaft m.b.H., Leipzig.

Nichols, P. B., D. R. Nelson & J. R. Garey (2006). A family level analysis of tardigrade phylogeny. Hydrobiologia 558: 53–60.

Plate, L. (1889). Beiträge zur Naturgeschichte der Tardigraden. Zoologische Jahrbücher, Anatomie und Ontogenie 3: 487–550.

Rahm, G. (1937). A new ordo of tardigrades from the hot springs of Japan (Furu-yu section, Unzen). Annotationes Zoologicae Japonenses 16: 345–352.

Sands, C. J., S. J. McInnes N. J., Marley, W. P. Goodall-Copestake, P. Convey & K. Linse (2008). Phylum Tardigrada: an "individual" approach. Cladistics 24: 861–871.

Schmidt-Rhaesa, A. (2001). Tardigrades – Are they really miniaturized dwarfs? Zoologischer Anzeiger 240: 549–555.

Schmidt-Rhaesa, A. & J. Kulessa (2007). Muscular architecture of *Milnesium tardigradum* and *Hypsibius* sp. (Eutardigrada, Tardigrada) with some data on *Ramazottius oberhaeuseri*. Zoomorphology 126: 265–281.

Schmidt-Rhaesa, A., T. Bartolomaeus, C. Lemburg, U. Ehlers & J. R. Garey (1998). The position of the Arthropoda in the phylogenetic system. Journal of Morphology 238: 263–285.

Shaw, K. (1974). The fine structure of muscle cells and their attachments in the tardigrade *Macrobiotus hufelandi*. Tissue & Cell 6: 431–445.

Walz, B. (1973). Zur Feinstruktur der Muskelzellen des Pharynx-Bulbus von Tardigraden. Zeitschrift für Zellforschung 140: 389–399.

— (1974). The fine structure of somatic muscles of Tardigrada. Cell & Tissue Research 149: 81–89.

Front cover photograph
Nanaloricus mysticus was the first species of Loricifera to be described.
Loricifera are now recognized as an abundant and typical element of diverse marine meiofaunal habitats.
Photo kindly by Reinhardt Møbjerg Kristensen, Copenhagen, Denmark.