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COMPARATIVE POLYAMINE ANALYSIS OF INVERTEBRATES: ROTIFERS, PLANARIANS, SPIDERS, CRUSTACEANS AND INSECTS

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ABSTRACT

The more than 35 biogenic polyamines have important roles in physiological processes ranging from acid-base buffering to the scavenging of oxygen free radicals. As such they have key cellular- and organismal-level functions in cell growth, cell differentiation, metamorphosis, spermatogenesis and fertilization in multicellular organisms. To determine cellular polyamine distribution profiles in animals at the base of the phylogenetic tree, the acid-extracted polyamines from 37 kinds of invertebrates (egg, larva, pupa and/or adult whole body) collected from various environments, were quantitatively analyzed using high-performance liquid chromatography and their concentrations (μmol /g wet weight) were calculated. Two rotifers (phylum Rotifera) contained putrescine, spermidine, homospermidine and spermine. Two land planarians (phylum Platyhelminthes) as well as freshwater planarians and marine planarians contained putrescine, cadaverine, spermidine and spermine. Diaminopropane, putrescine, spermidine and spermine were commonly distributed, while distribution of homospermidine, norspermidine and norspermine were taxonomically varied in daphnia, tadpole shrimp, brine shrimp, shrimp, opossum shrimp and isopod (subphylum Crustacea of phylum Arthropoda). In the class Insecta of the subphylum Hexapoda of phylum Arthropoda, aminopropylhomospermidine was detected in silk moths, stag beetles and rhinoceros beetles, and caldopentamine in mealworms.

Keywords: Arthropod, Insect, Invertebrate, Polyamine, Planarian, Rotifer

1. INTRODUCTION

More than 35 kinds of natural biogenic polyamines (linear diamines, triamines, tetra-amines, penta-amines and hexa-amines, and tertiary and quaternary branched triamines, tetra-amines and

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penta-amines, and guanidinoamines) can act as a controller for acid/base-buffering function and a free radical scavenger and can interact with various acidic biomolecules in cells, and therefore have various respective functions in the adaptation to environment for cell growth and the differentiation of cells to multicellular animals (Hamana et al., 1989, 1991a, 1991b, 1995, 2004, 2025a) and seed plants (Hamana et al., 2025b). To consider the phylogenetic and physiological significance of cellular polyamine distributions in invertebrates, we reported first the changes in polyamine levels in various organs of the silk moth *Bombyx mori*, an insect (Hamana et al., 1984), furthermore we systematically analyzed polyamines of other invertebrates belonging to the phyla Cnidaria, Nematode, Platyhelminthes, Annelida, Mollusca, Echinodermata and Arthropoda, and the subphylum Tunicata of the phylum Chordata (Hamana et al., 1989, 1991a, 1991b, 1995, 2004). Polyamines of sponges of the phylum Porifera, comb jelly of the phylum Ctenophora, and hydra, jellyfishes, sea anemones and soft corals of the phylum Cnidaria were analyzed in the most recent study (Hamana et al., 2025a).

Two rotifers (Rotifera), as a lower micro-invertebrate and two land planarians (Platyhelminthes) were available, and their polyamines were analyzed in the present study. A large invertebrate phylum, Arthropoda, had been divided into the four subphyla Chelicerata, Myriapoda, Crustacea and Hexapoda (classes Insecta and Entognatha) (Dunn et al., 2008; Telford et al., 2015) (Table 1), however, recently their classifications were reported by others (Regier et al., 2010; Rota-Stabelli et al., 2011). The class Insecta is divided traditionally into the subclasses Apterygota and Pterygota (recently the subclasses Monocondylia and Dicondylia) (Table 1). In addition to the previous polyamine analyses of some insects and spiders (Hamana et al., 1884, 1989, 1991b, 2004), polyamines of daphnias, tadpole shrimp, brine shrimp, shrimps, opossum shrimps and isopods belonging to the subphylum Crustacea were first analyzed and polyamines of new members of moth, cricket, beetle, fly and cockroach belonging to the subphylum Hexapoda, were analyzed in our present final study.

2. MATERIALS AND METHODS

Some invertebrate species were collected from markets or natural habitats without controlling for diet and growth conditions. Eggs, larvae, pupae and/or adult whole bodies of invertebrates (total 10 g-100 g wet weight) were homogenized in 5-10 % perchloric acid (PCA) by a mixer (Hamana et al., 2025a, 2025b). The PCA extract separated by a centrifugation (Hamana et al., 2025a, 2025b) was added to a column containing a cation-exchange resin (Hamana et al., 2004, 2025a, 2025b), and polyamines were eluted by 6M HCl to concentrate into the polyamine fraction. For analysis of the 35 kinds of biogenic polyamines, we have developed a combination of high-performance liquid chromatography (HPLC) and normal gas chromatography-mass spectrometry (GC-MS) (or high-performance GC-MS (HPGC-MS)). In the present study, the polyamine fraction was analyzed by HPLC on a Hitachi L6000 using a column of cation-exchange resin, using post-labeled

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fluorometry with *o*-phthalaldehyde (Hamana et al., 1995, 2004, 2025a, 2025b) (Fig. 1) because the contents of polyamines in the present polyamine fractions were too little for GC analysis. Since we have many data for polyamine analyses in our previous studies, we were able to identify and validate polyamines without GC-MS or HPGC-MS. Molar concentrations of polyamines (abbreviations and numeric cords for the number of methylene chain unit between N) per gram of wet weight of the invertebrates were roughly estimated by HPLC, using standard polyamines chemically synthesized in our laboratories (Niitsu et al., 1986, 1992) as shown in Table 1.

3. RESULTS AND DISCUSSION

3.1 Polyamines of rotifers

Rotifers are multicellular micro invertebrates and around 0.1–0.5 mm long. The freshwater rotifer *Branchionus calyciflorus* (20 g) and the marine rotifer *Branchionus plicatilis* (20 g) were purchased from an animal food market in Japan. The former freshwater species contained diaminopropane (3), putrescine (4), spermidine (34), homospermidine (44) and spermine (343), and the latter marine species contained diaminopropane, putrescine, norspermidine (33), spermidine, homospermidine and spermine (Fig. 1 and Table 1). Since the two species were cultured under same growth condition except for freshwater or seawater by the cultivator, the occurrence of norspermidine and a higher polyamine level is possibly dependent on salt condition. This is the first report of common occurrence of homospermidine in the phylum Rotifera.

3.2 Polyamines of planarians

In the phylum Platyhelminthes, our polyamine analyses of 16 species of the class Turbellaria, 2 species of the class Cestoda and 3 species of the class Trematoda by HPLC and GC-MS have been reported (Hamana et al., 1995, 1997, 2006), and some are cited in Table 1. After cultivation using chicken liver as bait (not containing norspermidine (33), homospermidine (44) and norspermine (333)) in our laboratory, the freshwater planarians *Dugesia japonica* collected in Haruna (Gunma), Himeji (Hyogo) and Taiwan, and *D. tigrina* collected in USA, and *D. ryukyuensis* collected in Okinawa, had the same polyamine profile (Hamana et al., 1995, 1997, 2006). Without cultivation in the present study, polyamines were extracted from the two land planarians (*Bipalium fuscatum*) (20-30 cm length) collected in Kiryu, Gunma, Japan (as No.1) and Tsumagoi, Gunma, Japan (as No.2) and then analyzed by HPLC. The former contained putrescine (4), cadaverine (5), spermidine (34) and spermine (343) and the latter contained diaminopropane (3), putrescine, cadaverine, spermine, homospermidine (44) and spermine (Table 1). Norspermidine (33) and norspermine (333) have been detected in two other land planarians *B. kewense* collected in Ogasawara Island (Tokyo) and *B. mobile* collected in Shibukawa (Gunma) analyzed without cultivation (Hamana et al., 2006) (Table 1). Effect of eating prey in their growth environments is not exclusive to the heterogenous polyamine profiles of *Bipalium* species. Norspermidine and

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norspermine were not found in other six species of triclads (freshwater planarians), five species of polyclads (marine planarians), two species of cestodes (tapeworms) and three species of trematodes (Hamana et al., 2006). Heterogeneous distribution of homospermidine was observed within the three classes of Platyhelminthes.

3.3 Polyamines of spiders

In subphylum Chelicerata in arthropods, polyamines of the whole body of the spider *Nephila clavate* belonging to the class Arachnida have been analyzed by HPLC and GC-MS in a previous study (Hamana et al., 1991b) and calculated in the present report, showed the occurrence of cadaverine (5) and its aminopropyl derivatives such as aminopropylcadaverine (35), aminopentylnorspermidine (335) and bis(aminopropyl)cadaverine (353) in addition to diaminopropane (3), norspermidine (33), norspermine (333) and caldopentamine (3333) (Table 1). In the polyamines of the silk gland (major ampullate gland) and head of another spider, *Araneus ventricosus,* analyzed by HPLC, we detected cadaverine but not the three cadaverine derivatives; aminopropylcadaverine, aminopentylnorspermidine and bis(aminopropyl)cadaverine (Hamana et al., 1991b) (Table 1). These polyamines have been detected as polycationic moieties of various types of spider venoms, as described in Hamana et al., 1991b. Since acidic phosphate ions are essential for spider silk production in major ampullate gland, basic polyamines must support the spider silk production.

3.4 Polyamines of crustaceans

In subphylum Crustacea in arthropods, two micro daphnias (*Moina macrocarpa* and *Daphnia magna*) purchased from an animal food market in Japan, contained high levels of diaminopropane (3), putrescine (4), cadaverine (5), spermidine (34), and low levels of homospermidine (44) and spermine (343) (Fig. 1) (Table 1). Eggs of tadpole shrimp (*Triops longicaudatus*), and eggs of brine shrimp (*Artemia franciscana*) were purchased from an aqua shop, Japan. We collected the adults of tadpole shrimp in Maebashi, Gunma, Japan. Nauplius larvae of the brine shrimp were obtained after the cultivation of the eggs in seawater in our laboratory. They contained diaminopropane, putrescine, cadaverine spermidine and spermine. Their eggs were poor in spermidine and spermine levels. Norspermidine (33) and norspermine (333) were not detected in the three orders of the class Branchiopoda of the subphylum Crustaceae (Table 1). In the class Malacostraca, dried samples of shrimps (*Lucensosergia lucens* and *Acetes japonicus*) and opossum shrimp (*Euphrasia pacifica*) were purchased from a food market in Maebashi. Isopods (*Armadillidium vulgare* and *Porcellionides pruinosus*) were collected in the garden of Maebashi Institute of Technology, Maebashi. In addition to common polyamines, norspermidine and norspermine were detected in them. Homospermidine was detected in the two terrestrial isopods

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(Fig. 1 and Table 1). Homospermidine was found specifically in the isopods living on the surface within the five orders Notostraca, Anostraca, Decapoda, Euphausiacea and Isopoda.

3.5 Polyamines of insects

In subphylum Hexapoda in arthropods, it has been reported that diaminopropane (3), norspermidine (33), norspermine (333) and thermospermine (334) in addition to common polyamines were found in three apterygotan insects (wingless and non-metamorphosing insects) belonging to the subclass Apterygota of the class Insecta of the subphylum Hexapoda by HPLC and GC-MS analyses in Hamana et al., 2004 (Table 1). In the subclass Pterygota of the class Incecta, we have analyzed polyamines of whole mature larvae of *Bombyx mori*, a silk moth (silkworm) and whole adults of *Periplanas americana*, a cockroach, by HPLC and GC-MS in Hamana et al., 1991b and calculated their polyamine concentrations in the present study (Table 1). We have HPLC-analyzed larvae, pupae and adult organs of crickets (*Teleogryllus emma*, G*ryllus bimaculatus* and *Homeogryllus japonicus*), cockroach (*Periplanas fuliginosa*), fly *(Drosophila melanogaster*) and midge (*Orthocladius akamushi*) in Hamana et al., 1989 and silk moth (*Antheraea yamamai* and *Galleria mellonella*) in Hamana et al., 1991b. Changes in polyamine level in various organs of *Bombyx mori* life cycle were reported by HPLC analysis (Hamana et al., 1984). In the present study, stag beetles (*Prosopocoilus inclinatus*) and rhinoceros beetles (*Trypoxylus dichotomus*) belonging to the subclass Pterygota were collected in Tsumagoi, Gunma, and pupae of *B*. *mori*, adults of crickets (*Acheta domesticus* and *Gryllus bimaculatus*), mealworms of a beetle (*Tenebrio molitor*), maggots (larvae) of housefly (*Musca domestica*) and adults cockroaches (*Blaptica dubia* and *Shelfordella lateralis*) were purchased from insect shops, insect feed markets and insect food markets in Japan, and their polyamines were analyzed by HPLC. The larva of the silk moth, larva and pupa of mealworm, larva of housefly, and larvae and pupae of rhinoceros beetle had various levels of cadaverine. It has been suggested that changes of cadaverine level related to the metamorphosing in silk moth (Haman et al., 1984). Although aminopropylhomospermidine (344) has not been detected in the two spiders previously analyzed, this tetra-amine was detected in some insects (Fig 1) (Table 1). Caldopentamine (3333) was found in the larva and pupa of mealworm in the present study as well as in an adult spider and an adult cockroach, as previously reported (Hamana et al., 1991b). Higher diamine and triamine levels were found in the males (whole body) of two beetles than their levels in the females (Table 1), supported by the different polyamine profiles within the sex-specific organs (male testis and female ovary) of silkworm, adult cricket and adult cockroach in their male spermatogenesis and female oogenesis (Hamana et al., 1984, 1989).

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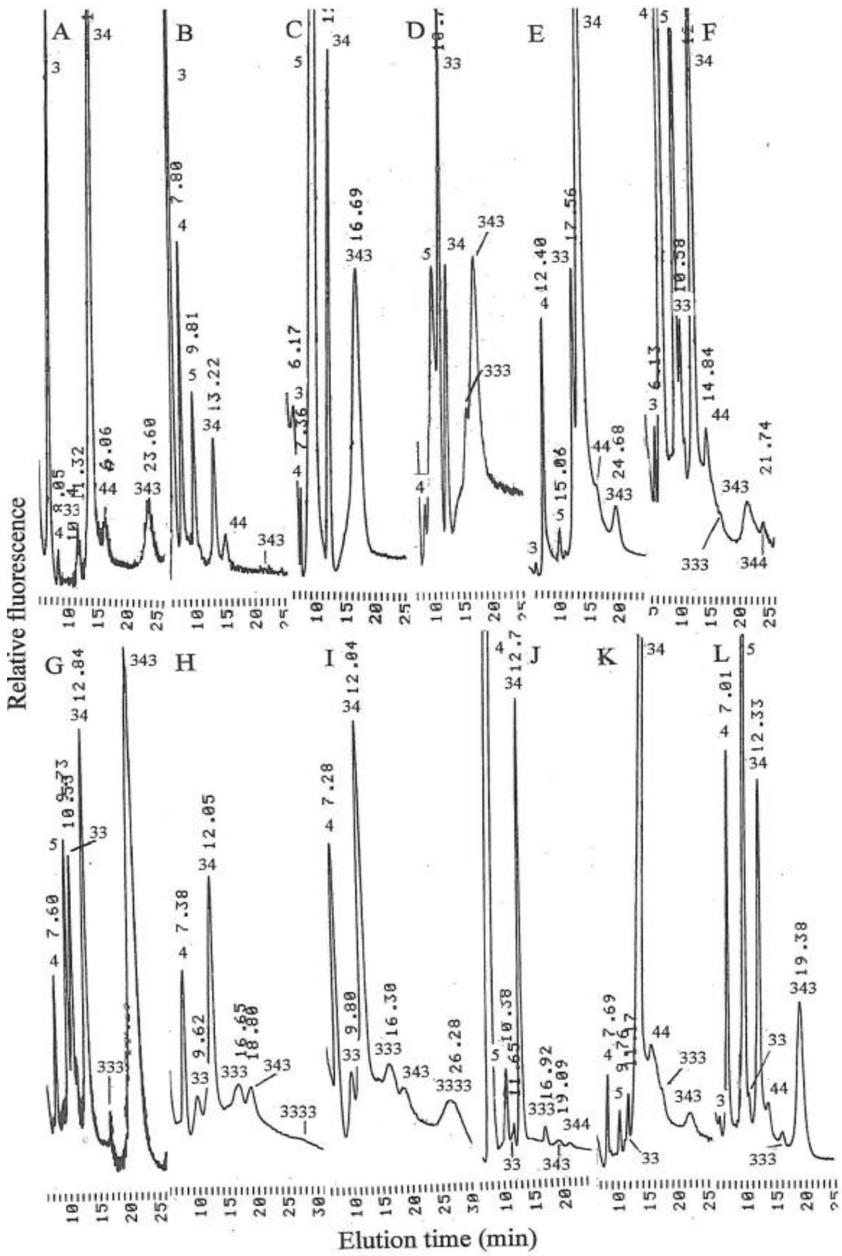

Fig. 1: HPLC charts of the polyamine fractions extracted from rotifer *Branchionus plicatilis* (A), daphnia *Moina macrocarpa* (B), tadpole shrimp *Triops longicaudatus* (adult) (C), shrimp *Lucensosergia lucens* (adult) (D), pill bug *Armadillidium vulgare* (adult) (E), silkworm *Bombyx mori* (pupa) (F), cricket *Gryllus bimaculatus* (adult) (G), mealworm *Tenebrio molitor* (larva) (H), mealworm *Tenebrio molitor* (pupa) (I), rhinoceros beetle *Trypoxylus dichotomus* (adult male) (J), maggot *Musca domestica* (larva) (K), and rusty red cockroach *Shelfordella lateralis* (adult male) (L). Since elution profiles of polyamines changed under unstable column and elution conditions, polyamine peaks were qualitatively identified and quantitatively determined by the coelution of chemically synthesized polyamine standards. Abbreviations for polyamines are shown in Table 1.

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Table 1: Cellular polyamine concentrations in rotifers, planarians and arthropods

Taxonomy and Organisms	References	Polyamines (μ mol/g wet weight)																	
Unranked Metazoa																			
Pylum Rotifera		Dap	Put	Cad	NSpd	Spd	APCad	HSpd	NSpm	Spm	TSpm	APHSpd	Can	HSpm	APNSpd	BAPCad	CPen	CHex	Agm
Class Rotatoria (rotifers)		3	4	5	33	34	35	44	333	343	334	344	434	444	335	353	3333	33333	
Branchionus calyciflorus (freshwater)		0.10	0.04	-	-	0.25	-	0.05	-	0.56	-	-	-	-	-	-	-	-	-
Branchionus plicatilis (marine)		1.30	0.10	-	0.14	1.55	-	0.10	-	0.15	-	-	-	-	-	-	-	-	-
Phylum Platyhelminthes																			
Class Turbellaria		Dap	Put	Cad	NSpd	Spd	APCad	HSpd	NSpm	Spm	TSpm	APHSpd	Can	HSpm	APNSpd	BAPCad	CPen	CHex	Agm
Order Tricladida (tricladida) (planarians)		3	4	5	33	34	35	44	333	343	334	344	434	444	335	353	3333	33333	
Dugesia japonica (freshwater planarian)	Hamana et al., 1995	-	1.40	0.04	-	3.14	-	0.08	-	1.29	-	-	-	-	-	-	-	-	0.02
Dugesia ryukyuensis (freshwater planarian)	Hamana et al., 2006	-	1.60	0.70	-	0.90	-	0.02	-	0.44	-	-	-	-	-	-	-	-	-
Dugesia tigrina (freshwater planarian)	Hamana et al., 1997	-	1.19	0.05	-	0.02	-	0.17	-	0.46	-	-	-	-	-	-	-	-	0.01
Bipalium fuscatum (land planarian) No. 1		-	0.70	0.22	-	0.60	-	-	-	0.85	-	-	-	-	-	-	-	-	-
No. 2		0.21	0.50	0.15	-	0.53	-	0.15	-	0.90	-	-	-	-	-	-	-	-	0.05
Bipalium kewense (land planarian)	Hamana et al., 2006	-	0.55	0.02	0.02	1.40	-	-	0.10	0.30	-	-	-	-	-	-	-	-	-
Bipalium nobile (land planarian)	Hamana et al., 2006	0.12	0.95	-	0.03	0.30	-	0.30	0.25	0.86	-	-	-	-	-	-	-	-	-
Platydemus manokwari (land planarian)	Hamana et al., 2006	-	0.44	0.27	-	0.35	-	0.02	0.04	0.75	-	-	-	-	-	-	-	-	-
Order Polycladida (polyclads) (flatworms)																			
Notoplana humilis (marine planarian)	Hamana et al., 2006	0.08	0.15	0.20	-	0.40	-	-	-	0.30	-	-	-	-	-	-	-	-	-
Class Cestoda (cestodes) (tapeworms)																			
Hymenolepis diminuta	Hamana et al., 2006	-	-	0.02	-	0.45	-	0.02	-	0.87	-	-	-	-	-	-	-	-	-
Class Trematoda (trematodes)																			
Paragonimus westermanii	Hamana et al., 2006	-	-	0.05	-	0.30	-	-	-	0.20	-	-	-	-	-	-	-	-	-
Phylum Arthropoda (arthropods)																			
Subphylum Chelicerata		Dap	Put	Cad	NSpd	Spd	APCad	HSpd	NSpm	Spm	TSpm	APHSpd	Can	HSpm	APNSpd	BAPCad	CPen	CHex	Agm
Class Arachnida (spiders)		3	4	5	33	34	35	44	333	343	334	344	434	444	335	353	3333	33333	
Nephila clavata adult whole body	(calculated)	0.02	0.66	0.02	0.24	1.20	0.03	-	0.64	0.80	0.12	-	-	-	0.02	0.04	0.04	-	0.50
adult silk gland	Hamana et al., 1991b	0.01	2.60	0.06	0.24	2.50	-	-	1.08	0.10	-	-	-	-	-	-	-	-	0.12
adult head	Hamana et al., 1991b	0.10	1.45	0.12	0.38	7.00	-	-	0.53	0.86	-	-	-	-	-	-	-	-	0.30
Araneus ventricosus adult silk gland	Hamana et al., 1991b	0.12	0.47	0.40	0.40	2.50	-	-	0.14	0.22	-	-	-	-	-	-	-	-	0.25
adult head	Hamana et al., 1991b	0.04	0.25	0.40	0.50	3.50	-	-	0.86	0.70	-	-	-	-	-	-	-	-	0.25
[Subphylum Myriapoda not available]																			
Subphylum Crustacea (crustarceans)																			
Class Branchiopoda		Dap	Put	Cad	NSpd	Spd	APCad	HSpd	NSpm	Spm	TSpm	APHSpd	Can	HSpm	APNSpd	BAPCad	CPen	CHex	Agm
Order Diplostraca (daphnias) (water fleas)		3	4	5	33	34	35	44	333	343	334	344	434	444	335	353	3333	33333	
Moina macrocarpa (*M. macrocopa*) (freshwater)		1.35	0.67	0.35	-	0.27	-	0.07	-	0.01	-	-	-	-	-	-	-	-	-
Daphnia magna (freshwater)		0.80	0.75	0.05	-	0.10	-	0.01	-	0.02	-	-	-	-	-	-	-	-	-
Order Notostraca																			
Triops longicaudatus (tadpole shrimp) diapase egg		-	0.18	0.10	-	0.05	-	-	-	0.04	-	-	-	-	-	-	-	-	-
(freshwater) adult whole body		0.05	0.16	1.90	-	1.15	-	-	-	0.75	-	-	-	-	-	-	-	-	-
Order Anostraca																			
Artemia franciscana (brine shrimp) diapase egg		0.03	0.70	0.05	-	0.45	-	-	-	-	-	-	-	-	-	-	-	-	-
(marine) nauplius larva		-	0.10	0.10	-	0.75	-	-	-	0.18	-	-	-	-	-	-	-	-	-
Class Malacostraca																			
Order Decapoda (shrimps)																			
Lucensosergia lucens		-	0.05	0.60	1.20	0.60	-	-	0.10	0.53	-	-	-	-	-	-	-	-	-
Acetes japonicus		-	-	0.04	0.90	0.60	-	-	0.06	0.16	-	-	-	-	-	-	-	-	-
Order Euphausiacea (opossum shrimps)																			
Euphausia pacifica		-	-	0.82	0.33	0.30	-	-	0.10	1.90	-	-	-	-	-	-	-	-	-
Order Isopoda (isopods)																			
Armadillidium vulgare (pill bug)		0.02	0.55	0.05	0.50	1.90	-	0.02	-	0.10	-	-	-	-	-	-	-	-	-
Porcellionides pruinosus (wood louse)		-	0.01	0.02	0.52	0.06	-	0.08	0.08	0.08	-	-	-	-	-	-	-	-	-
Subphylum Hexapoda																			
[Class Entognatha not available]																			
Class Insecta (insects)		Dap	Put	Cad	NSpd	Spd	APCad	HSpd	NSpm	Spm	TSpm	APHSpd	Can	HSpm	APNSpd	BAPCad	CPen	CHex	Agm
Subclass Apterygota		3	4	5	33	34	35	44	333	343	334	344	434	444	335	353	3333	33333	
Sinella curviseta (springtail)	Hamana et al., 2004	-	0.13	0.72	0.01	0.92	-	-	0.02	-	-	-	-	-	-	-	-	-	-
Pedetontus nipponicus (bristeltail)	Hamana et al., 2004	0.08	0.13	0.15	0.09	0.37	-	-	0.20	-	-	-	-	-	-	-	-	-	-
Lepisma saccharina (silverfish)	Hamana et al., 2004	0.01	0.09	0.04	0.08	1.05	-	-	0.06	0.06	-	-	-	-	-	-	-	-	-
Subclass Pterygota																			
Order Orthptera (crickets)																			
Acheta domesticus adult		-	0.15	0.60	0.68	0.53	-	-	0.03	0.34	-	-	-	-	-	-	-	-	-
Gryllus bimaculana adult		-	0.35	0.62	0.60	0.91	-	-	0.03	1.17	-	-	-	-	-	-	-	-	-
testis	Hamana et al., 1989	-	0.01	-	0.01	0.16	-	-	0.39	0.08	-	-	-	-	-	-	-	-	-
ovary	Hamana et al., 1989	-	0.06	-	0.10	0.51	-	-	0.64	0.01	-	-	-	-	-	-	-	-	-
Order Blattodea (cockroaches)																			
Periplanas americana (american cockroach) adult	(calculated)	0.10	0.34	0.10	0.37	0.13	-	0.08	1.50	0.22	0.05	0.02	-	0.02	-	-	0.21	0.04	-
testis	Hamana et al., 1989	-	0.10	-	-	0.13	-	-	0.27	0.03	-	-	-	-	-	-	-	-	-
ovary	Hamana et al., 1989	0.01	0.01	-	0.10	0.02	-	-	0.24	-	-	-	-	-	-	-	-	-	-
Blaptica dubia (dubia roach) adult male		0.02	0.44	0.40	0.20	0.55	-	0.02	0.03	0.03	-	-	-	-	-	-	-	-	-
adult female		0.02	0.85	1.40	0.25	0.56	-	0.04	0.02	0.05	-	-	-	-	-	-	-	-	-
Shelfordella lateralis (*Blatta lateralis*) adult male		0.02	0.85	1.35	0.10	0.80	-	0.05	0.03	0.35	-	-	-	-	-	-	-	-	-
(rusty red cockroach) adult female		-	0.98	0.65	0.10	0.82	-	0.04	0.03	0.04	-	-	-	-	-	-	-	-	-
Order Coleoptera (beetles)																			
Tenebrio molitor (mealworm) larva		-	0.35	-	0.04	0.53	-	-	0.06	0.05	-	-	-	-	-	-	0.01	-	-
pupa		-	0.55	-	0.10	0.82	-	-	0.05	0.03	-	-	-	-	-	-	0.10	-	-
Procopocoilus inclinatus (stag beetle) adult male		-	0.73	0.35	0.10	0.78	-	-	0.02	0.02	-	0.01	-	-	-	-	-	-	-
adult female		-	0.71	0.30	0.07	0.75	-	-	0.01	0.02	-	0.02	-	-	-	-	-	-	-
Trypoxylus dichotomus (rhinoceros beetle) larva male		-	4.12	0.23	0.10	0.80	-	0.03	0.02	0.02	-	0.01	-	-	-	-	-	-	-
larva female		-	2.05	0.02	0.03	0.47	-	0.02	0.01	0.02	-	0.01	-	-	-	-	-	-	-
pupa male		-	2.20	0.08	0.02	1.05	-	0.02	0.03	0.02	-	0.01	-	-	-	-	-	-	-
pupa female		-	1.80	0.07	0.02	0.42	-	-	0.02	0.02	-	0.02	-	-	-	-	-	-	-
adult male		-	2.16	0.37	0.10	1.02	-	-	0.03	0.02	-	0.02	-	-	-	-	-	-	-
adult female		-	1.42	0.16	0.03	0.59	-	0.01	0.03	0.02	-	0.02	-	-	-	-	-	-	-
Order Diptera (flies, mosquitoes)																			
Musca domestica (housefly) larva (maggot)		-	0.20	0.08	0.09	1.90	-	0.05	0.03	0.05	-	-	-	-	-	-	-	-	-
Order Lepidoptera (butterflies, moths)																			
Bombyx mori (silk moth) larva (silkworm)	(calculated)	0.03	0.27	-	0.08	1.52	-	0.01	0.18	0.33	-	-	-	-	-	-	-	-	-

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pupa		0.15	1.10	0.45	0.10	2.02	-	0.12	0.02	0.08	-	0.04	-	-	-	-	-	-	-
silk gland of larva	Hamana et al., 1984	0.03	2.08	-	0.03	3.18	-	0.04	0.11	0.46	-	-	-	-	-	-	-	-	-
testis of larva	Hamana et al., 1984	-	5.21	-	0.04	1.10	-	-	0.08	0.14	-	-	-	-	-	-	-	-	-
ovary of larva	Hamana et al., 1984	-	1.28	-	-	0.80	-	-	0.08	0.31	-	-	-	-	-	-	-	-	-
testis of moth	Hamana et al., 1984	0.03	10.5	-	0.43	0.89	-	-	0.11	0.10	-	-	-	-	-	-	-	-	-
ovary of moth	Hamana et al., 1984	-	1.54	-	0.03	0.36	-	-	0.05	0.06	-	-	-	-	-	-	-	-	-

Abbreviations for polyamines: Dap (3) (abbreviations for the number of methylene chain unit between N), diaminopropane; Put (4), putrescine; Cad (5), cadaverine; NSpd (33), norspermidine; Spd (34), spermidine; APCad (35), aminopropylcadaverine; HSpd (44), homospermidine; NSpm (333), norspermine; Spm (343), spermine; TSpm (334), thermospermine; APHSpd (344), aminopropylhomospermidine; Can (434), canavalmine; HSpm (444), homospermine (aminobutylhomospermidine); APNSpd (335), aminopentylnorspermidine; BAPCad (353), bis(aminopropyl)cadaverine; CPen (3333), caldopentamine; CHex (33333), caldohexamine; -, not detected (＜0.005 μmol/g wet weight). [ ], organism belonging to the phylum, subphylum and class was not available in our studies. Some our data previously published are cited in References, and blanks in References are new data in the present study. Concentrations of polyamines of *Nephila clavate* (whole body), *Bombyx mori* (larva) and *Periplanas americana* (whole body) were calculated from the HPLC and GC-MS charts of the previous report (Hamana et al., 1991b) and are shown in this table as (calculated).

3.6 Polyamines of invertebrates and vertebrates

The present report is our final report on polyamine distribution profiles in the 11 major invertebrate phyla within the 34 total phyla of Animalia (Metazoa), in comparisons with our polyamine analyses of vertebrates belonging into the phylogenetically top subphylum Vertebrata of the phylum Chordata within the 34 phyla. It had been shown first that putrescine (Put) (4), spermidine (Spd) (34) and spermine (Spm) (343) were the major common polyamines in the mammalian pig (hog) thyroid and rat thyroid (Hamana et al., 1984; Matsuzaki et al., 1978, 1981a). In some organs of some fishes, amphibians, reptiles, birds and mammals, homospermidine (HSpd) (44), aminopropylhomospermidie (APHSpd or AHSpd) (344) and canavalmine (Can) (434) were detected in epididymides of the sexually mature male rodents such as syrian hamster, chinese hamster and guinea pig, but not found in other rodent epididymides of mouse, rat, mongolian gerbil and house musk shrew (Matsuzaki et al., 1982, 1989). Cadaverine (Cad) (5) and homospermidine (HSpd) (44) were found as a major polyamine in the testis of some amphibians and reptiles but not found in the testis of fishes and birds (Hamana and Matsuzaki, 1979; Matsuzaki et al., 1981b). In addition to homocaldopentamine (HCPen) (3334) and thermopentamine (TPen) (3343) found in a sea urchin and a sea cucumber belonging to the phylum Echinodermata and homospermine (HSpm) (444) found in a tunicate belonging to the second subphylum Tunicata of the phylum Chordata (Hamana et al., 1991a) and an insect (Table 1), norspermidine (NSpd) (33), norspermine (NSpm) (333), thermospermine (TSpm) (334), penta-amines and hexa-amines were not detected in the vertebrates. N^1-Acetylspermidine and N^1-acetylspermine were detected in rodent epididymides (Matsuzaki et al., 1982). We studied acetylpolyamine levels in mouse tissues and primary culture of adult rat hepatocytes (Sugimoto et al., 1988, 1989, 1992) and human HeLa cells

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(Ichimura et al., 1998, 2004). Acetylpolyamines were not detected in our invertebrate polyamine analyses.

Polyamine analysis of rotifers, daphnias, shrimps and insects is important for nutritional food science and contribute to aquaculture, animal husbandry and/or human health.

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