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Full Length Research Paper

Analysis of cavitating flow through a venturi

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A dynamical study of a bubbly flows in a transversal varying section duct (Venturi), is modeled by the use of the mass and momentum phases equations, which are coupled with the Rayleigh-Plesset equation of the bubbles dynamics. The effects of the throat dimension and the upstream void fraction on flow parameters are investigated. The numerical resolution of the previous equations set let us found that the characteristics of the flow change dramatically with upstream void fraction. Two different flow regimes are obtained: a quasi-steady and a quasi-unsteady regimes. The former is characterized by a large spatial fluctuations downstream of the throat, which are induced by the pulsations of the cavitation bubbles. The quasi-unsteady regime corresponds to flashing flow in which occurs a bifurcation at the flow transition between these regimes. This transition occurs at $R_c \approx$ 4.3 which corresponds to $\alpha_s > 4.710^{-3}$. An analytical expression for the critical bubble size at the flashing flow point is also obtained and compared with theoretical data.

Key words: Venturi meter, tow-phase flow, cavitation.

INTRODUCTION

It is well known that the venturi is a robust technique for measuring the flow characteristics of a single-phase fluid for high Reynolds numbers. Multiphase flow measuring is generally more difficult. The density of a gas-liquid mixture depends upon the volume fraction of the gas, and the phases densities. The velocity of the gas within the venturi is likely to be different from that of the liquid. Over the two last decades, the investigations of a homogeneous steady-state cavitating nozzle flow, using spherical bubble dynamics with a polytropic thermal process (Wang and Brennen, 1998), have shown some flow instabilities illustrated by the flashing flow phenomenon.

The flow model, generally used, is a nonlinear continuum bubbly mixture which is coupled with the dynamics equation of the bubbles. A three equations model was first proposed by van Wijngaarden (1968, 1972), and has been used for studying steady and transient shock wave propagation in bubbly liquids, by omitting the acceleration of the mean flow. This model has been also considered by Wang and Brennen (1998), in the case of converging-diverging nozzle, with an upstream variable void fraction. It was observed that significant change of the flow characteristics depends strongly on the latter and a critical bubbles radius have been obtained. Considering the gas nucleation rate, as a

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source term in the mass conservation equation of the bubbles, Delale et al. (2003) have used the previous model for the same converging-diverging nozzle. They have concluded that the encountered flow instability can be stabilised by thermal damping. Several authors have also considered the bubble dynamics equations under an appropriate form to the chosen example. Among them, Wang and Brennen (1999) have expressed the flow equations in time and radial coordinate, for a bubbly mixture, where the shock wave have been studied for spherical cloud of cavitating bubbles. Besides, effects of the shocks on the bubbles interactions have also been analysed. The same Rayleigh-Plesset equation has been used by Gaston et al. (2001) in modelling the bubbles as a potential source. The stream function has been written in function of spatial coordinates and the source term. They have analysed the effect of complex interactions through a venturi. By introducing liquid quantity and motion equation in a spatial Rayleigh-Plesset dynamics relation, Moholkar and Pandit (2001) have obtained a global dynamic equation which has been resolved in a three steps method. In their work they have studied the effect of the downstream pressure, the venturi pipe ratio, the initial bubble size and the upstream void fraction, on the dynamics of the flow. The results of the simulations show that the bubble/bubble and bubble/flow interaction through the hydrodynamic of the flow has important effect on the behaviour of the bubble flow. Considering a one bubble motion in a venturi, Soubiran and Sherwood (2000) have obtained a dynamic equation of the flow, based on different acting forces.

More recently, Ashrafizadeh and Ghassemi (2015) have experimentally and numerically investigate the effects of the geometrical parameters, such as throat diameter, throat length, and diffuser angle, on the mass flow rate, critical pressure ratio and application rang of small-sized cavitating venturis (CVs). The obtained results show that the CVs in very small size are also capable in controlling and regulating the mass flow rate while their characteristic curves are similar to those of ordinary CVs with larger throat sizes. Also, by decreasing the throat diameter of CVs, the choked mode region, the critical pressure and discharge coefficient decrease. By decreasing the diffuser angle from 15 to 5° in the numerical simulations, the critical pressure ratio increases and the discharge coefficient remains constant. By increasing the throat length of CVs, the critical pressure ratio decrease while discharge coefficient does not shown any changes. Also, a variable area cavitating venturi was designed and investigated experimentally by Tian et al (2014). Four sets of experiments were conducted to investigate the effect of the pintle stroke, the upstream pressure and downstream pressure as well as the dynamic motion of the pintle on the performance of the variable area cavitating venturi. The obtained results verify that the mass flow rate is independent of the downstream pressure when the downstream pressure ration is less about 0.8. The mass flow rate is linearly dependent on the pintle stroke and increases with the upstream pressure. The discharge coefficient is a function of the pintle stroke; however it is independent of the upstream pressure. They concluded that the variable area cavitating venturi can control and measure the mass flow rate dynamically.

Our investigation is based on the first model (a non linear continuum bubbly mixture model coupled with the dynamic equation of the bubble), the present work considers a cavitating flow through a venturi. The effect of the throat diameter of the venturi and the limits of flashing flow occurring for some upstream voids fraction, are analysed, and a critical value of bubble radius at the flashing flow point is obtained.

BASIC EQUATIONS

An axisymmetric venturi with cross-sectional area A(x) is showed in Figure 1, where the dimensions are reported to the inlet radius *a.* The liquid is assumed to be incompressible and the relative motion between the liquid and the duct wall is neglected and the total upstream bubble population is uniform without coalescence and further breaks up of the bubbles in the flow. Gas and vapor densities are neglected in comparison to one of the liquid. The bubbles are assumed to have the same initial radius R_S^*. Friction between the liquid and the duct wall is neglected and the relative motion between the tow phases ignored.
Then the mixture density can be expressed in function of bubble popula
tion

η: $\rho = \rho_L(1-\eta V)$

Where $V = 4/3\pi R^3(x,t)$ is the bubble volume.

Continuity and momentum equations of the bubbly flow (Wang and Brennen, 1998) are:

$$\frac{\partial}{\partial t}[(1-\alpha)A] + \frac{\partial}{\partial x}[(1-\alpha)uA] = 0 \quad (1)$$

$$\frac{\partial u}{\partial t} + u\frac{\partial u}{\partial x} = -\frac{1}{2(1-\alpha)}\frac{\partial Cp}{\partial x} \quad (2)$$

Where $\alpha(x,t) = 4/3\pi\eta R^3/\left[1+4/3\pi\eta R^3\right]$ is the bubble void fraction, and u(x, t) the fluid velocity.
$Cp(x,t) = \left(p^*(x,t) - p_s^*\right)/1/2\rho_L^* u_s^{*2}$ is the fluid pressure coefficient, and p(x, t) the fluid pressure, p_s^* the upstream fluid pressure, and u_s^* the upstream fluid velocity. The dynamics of the bubbles can be modeled by the Rayleigh-Plesset equation (Knapp et al., 1970; Daily and Hammitt, 1970; Plesset and Prosperetti, 1977).

$$R\frac{D^2R}{Dt^2} + \frac{3}{2}\left(\frac{DR}{Dt}\right)^2 + \frac{\sigma}{2}\left(1-R^{-3k}\right) + \frac{4}{Re}\frac{1}{R}\frac{DR}{Dt} + \frac{2}{We}\left(R^{-1}-R^{-3k}\right) + \frac{1}{2}Cp = 0 \quad (3)$$

Where $D/Dt = \partial/\partial t + u\partial/\partial x$ is the Lagrangian derivative,

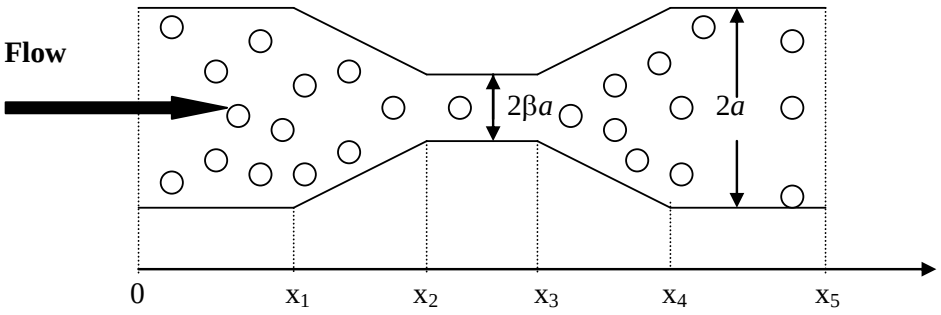

Figure 1. Schematic of the venture.

Table 1. Initial conditions and water characteristics .

Initial parameter	Water characteristics at 20°C
R_s^* =100 µm	ρ_L^* =1000 Kglm^3
u_s^* =10 m/s	μ_E^* =0.03 Ns/m^2
k=1.4	μ_L^* =0.001 Ns/m^2
Re=33	S*=0.073 N/m
σ=0.8	
We=137	

$\sigma = \left(p_s^* - p_v^*\right) / 1/2\rho_L^* u_s^2$ the cavitation number, p_v^* the partial pressure of vapor inside the bubble. $\mathrm{Re} = \rho_L^* u_s^* R_s^* / \mu_E^*$ is the Reynolds number, μ_E^* the effective viscosity of liquid. $\mathrm{We} = \rho_L^* u_s^{*2} R_s^* / S^*$ is the Weber number, S* represent the liquid surface tension, and ρ_L^* the liquid density.

Equations (1), (2) and (3) constitutes a simple model of one-dimensional two phase bubbly flow bubbly with a nonlinear bubble dynamics equation.

Steady-state solutions

Assuming steady-state conditions, all the partial time derivative terms in Equations (1) to (3) disappear. Then, Equation set (1) to (3) can be transformed into an ordinary differential equation set, with only one independent variable (x):

$$(1-\alpha)uA=(1-\alpha_s)=\text{constant} \tag{4}$$

$$u\frac{du}{dx} = -\frac{1}{2(1-\alpha)}\frac{dCp}{dx} \tag{5}$$

$$R\left(u^2\frac{d^2R}{dx^2} + u\frac{du}{dx}\frac{dR}{dx}\right) + \frac{3u^2}{2}\left(\frac{dR}{dx}\right)^2 + \frac{4}{Re}\frac{u}{R}\frac{dR}{dx} + \frac{2}{We}\left(\frac{1}{R} - \frac{1}{R^{3k}}\right) + \left(1 - \frac{1}{R^{3k}}\right) + \frac{1}{2}Cp = 0 \tag{6}$$

The corresponding initial conditions are:

$$R(x=0)=1, \quad U(x=0)=1, \quad Cp(x=0)=0 \tag{7}$$

The axial variation of the cross sectional takes the following from:

$$A(x) = \begin{cases} 1 & 0 < x < x_1 \\ 1 - \dfrac{(x - x_1)(1-\beta)}{x_2 - x_1} & x_1 < x < x_2 \\ \beta & x_2 < x < x_3 \\ 1 - \dfrac{(x_4 - x)(1-\beta)}{x_4 - x_3} & x_3 < x < x_4 \\ 1 & x_4 < x < x_5 \end{cases} \tag{8}$$

Where β is the dimensionless radius of the venturi throat, and x the distance along the axis. In the present work we assumed: β=0.5, x_1=3.0, x_2=5.7, x_3=6.7, x_4=10.5

RESULTS AND DISCUSSION

Equation set (4 to 6) is resolved by the use of a fourth order Runge-Kutta scheme, with some flow conditions (Table 1).

Venturi diameter throat effect

Three non-dimensional diameters ventuti throat (β) 0.5, 0.6 and 0.7 were tested numerically. Effect of the bubble radius evolution is showed in Figure 3 for some non-

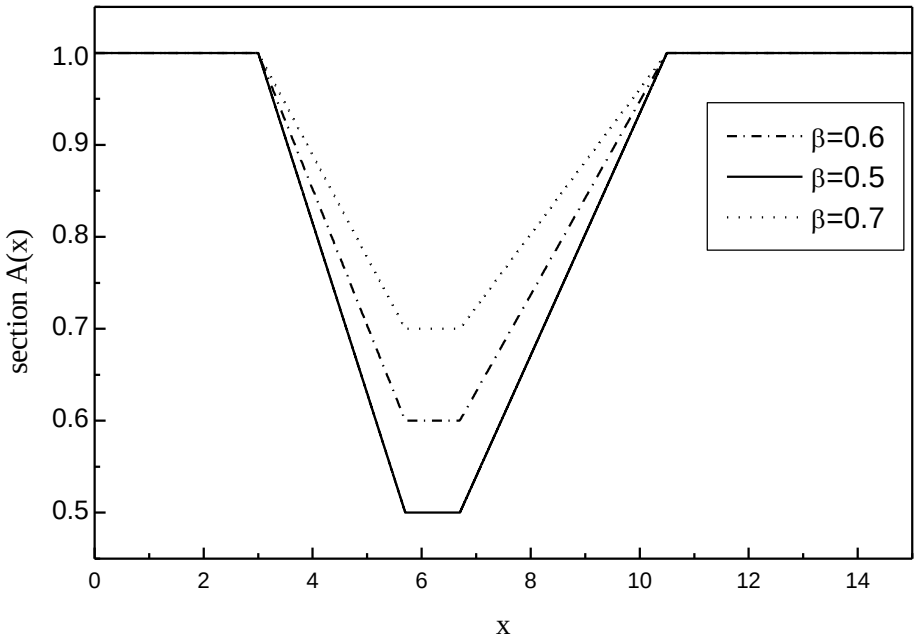

Figure 2. Venturi sections for various values of the non-dimensional throat radius β.

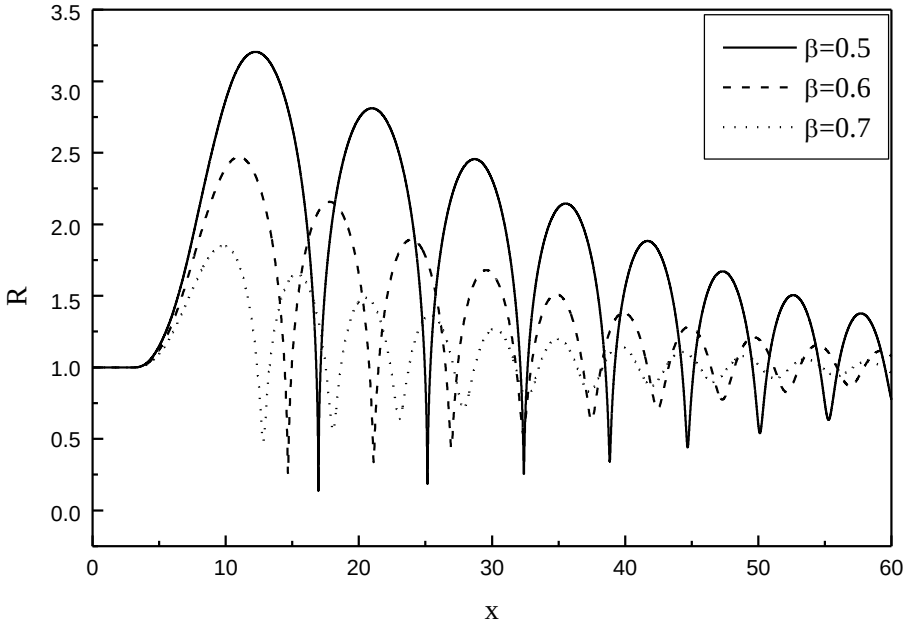

Figure 3. Bubbles radius for various values of the non-dimensional throat radius β.

dimensional diameters of the venturi throat (Figure 2). By increasing the diameters venturi throat, the bubble radius decrease and the bubble oscillations frequency increase. Fluid axial velocity and pressure distribution are drawn in Figures 4 and 5, for different throat diameters. It seems that the evolution of these parameters corresponds to the monophasic case (Figure 4). In the Figure 6, the axial bubble radius gradient gives a large value after the throat section which is due to the inertial phenomena, as explained in Blak (1949) work. A strongly dumping is also observed for the subsequent peaks. Figure 7, shows a part of the previous (Figure 6), corresponding to a small distance, where the continuity of radius gradient can be verified.

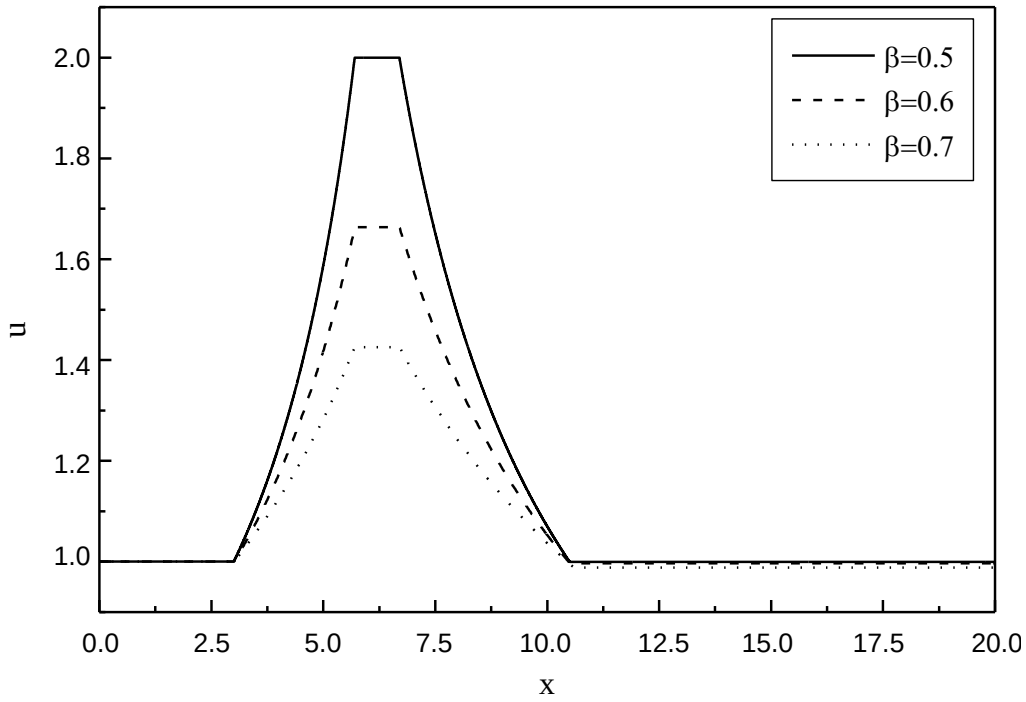

Figure 4. Fluid velocity for various values of the non-dimensional throat radius β.

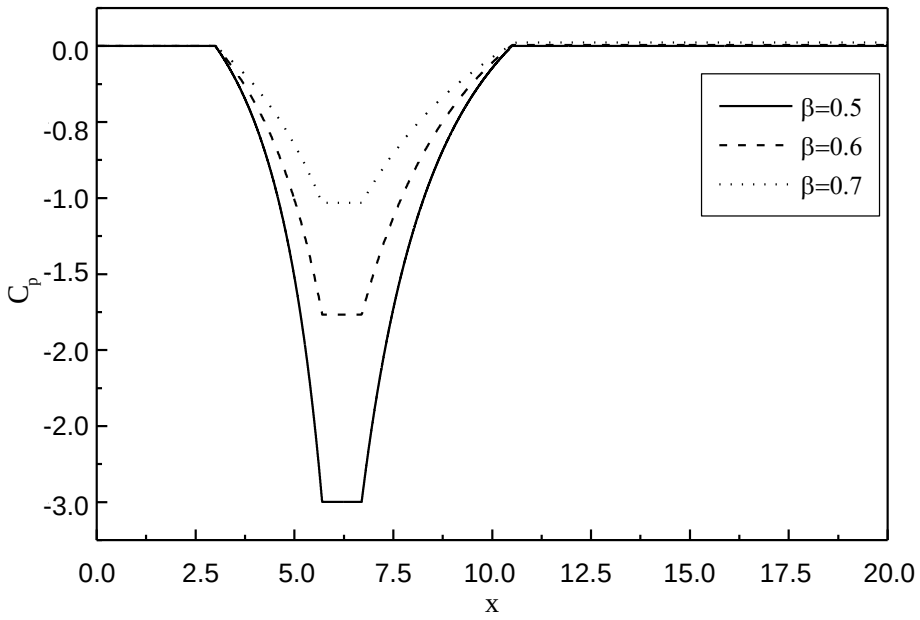

Figure 5. Fluid pressure for various values of the non-dimensional throat radius β.

Upstream void fraction effect

Five different upstream void fractions (α_s) of the order of 10^{-3} are used in the computation to study, the effect of the upstream void fraction on the flow structure through the ventuti. The case of α_s=0 corresponds to the incompressible pure liquid flow, the results are shown in Figures 8, 9 and 10 which correspond to the non-dimensional bubble radius distribution, fluid velocity and fluid pressure coefficient, respectively, an instability

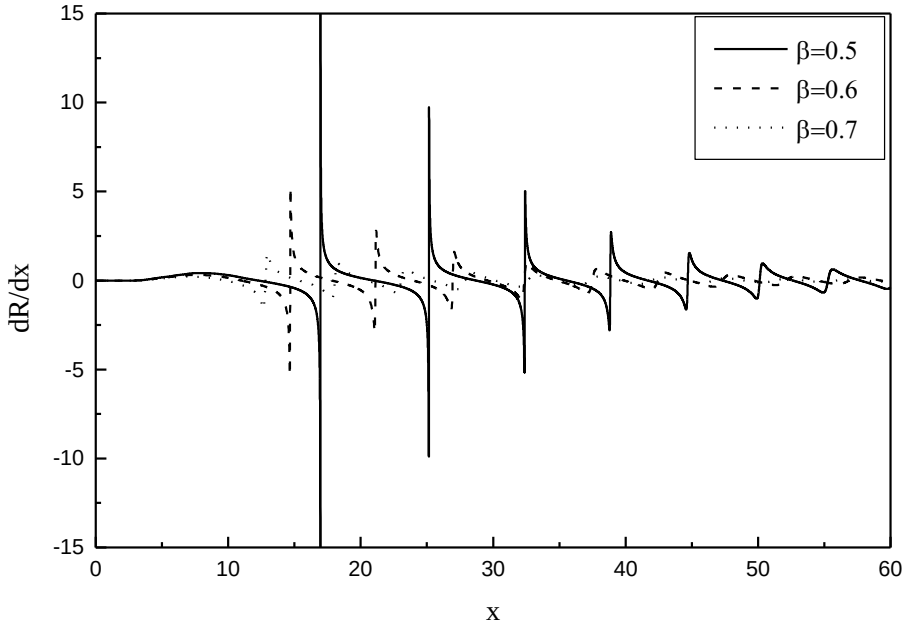

Figure 6. Bubble radius gradient for various values of the non-dimensional throat radius β.

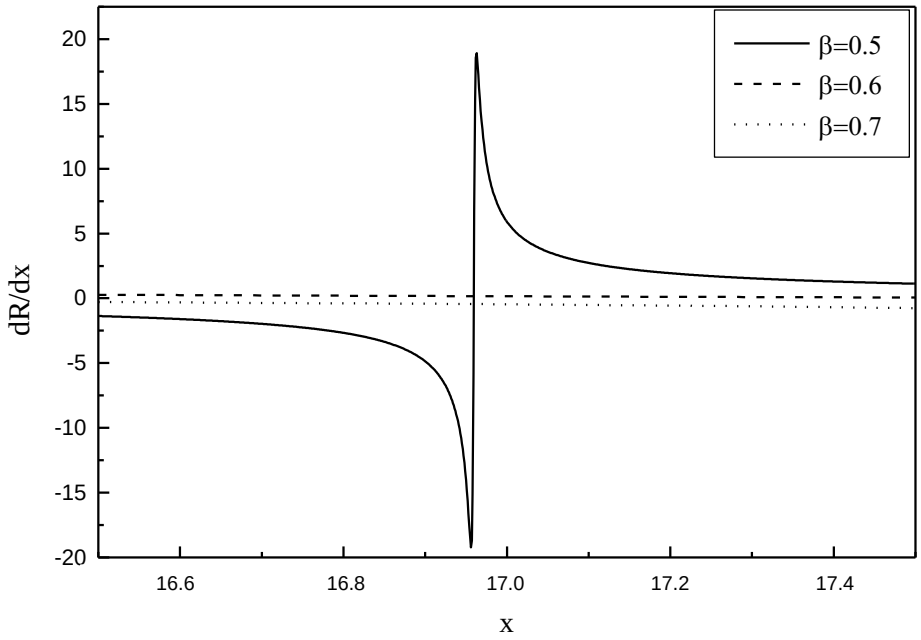

Figure 7. A part of Figure 6.

inception can be remarked in these figures, which is located just after the throat, these results confirm those of Wang and Brennen (1998) with no important differences.

Figure 8 shown that the bubble size reach the maximum after passing the nozzle throat of the venturi with increase in the upstream void fraction, the maximum size of the bubbles increases and bubble frequency oscillation decrease, this maximum size is shifted further downstream after it reach the critical radius (instability occurs), the bubbles growth without bound in the calculation, this instability occurs when the bubble reaches a critical value, also the void fraction growing

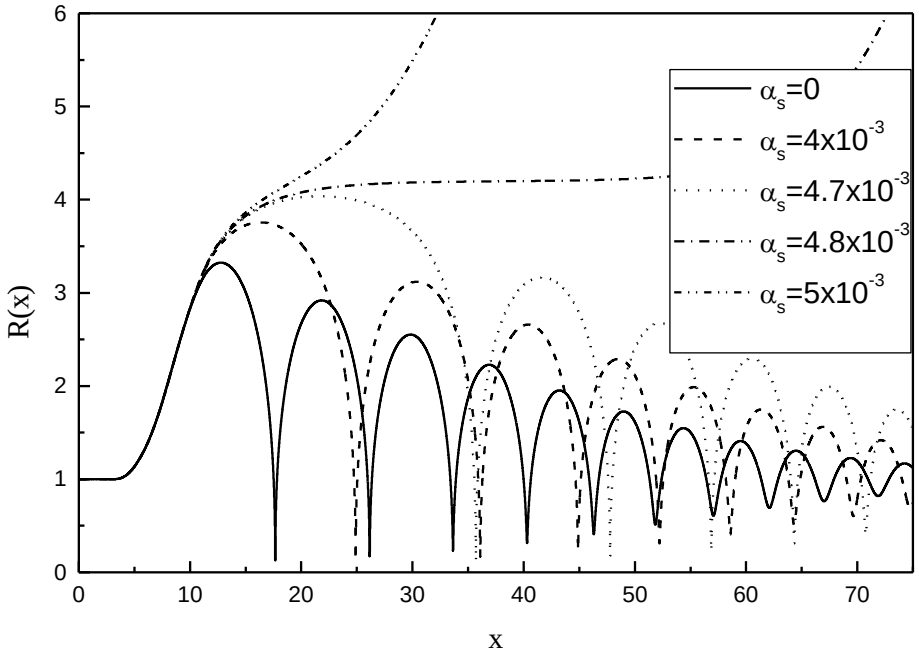

Figure 8. Axial bubble radius distribution for deferent upstream void fraction.

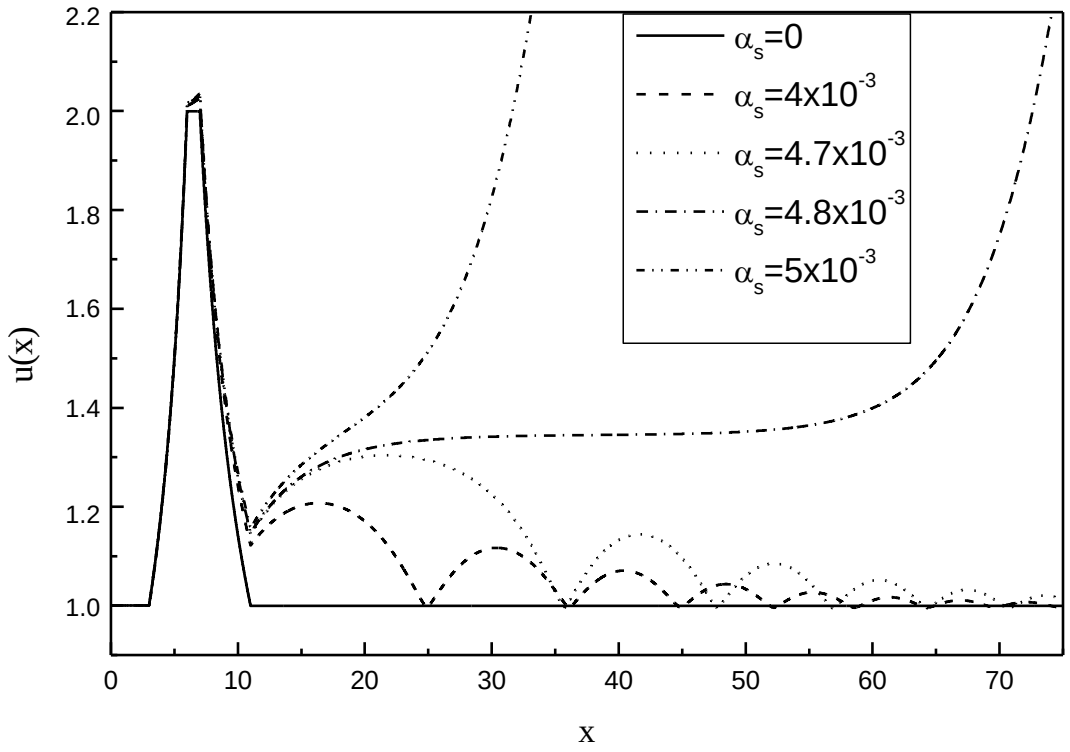

Figure 9. Axial fluid velocity distribution for different upstream void fractions.

leads to large amplitudes of the previously drown parameters, an important remark concerns the venturi geometry effect: in the Wang and Brennen (1998) work, where cavitation in converging-diverging nozzle bubbly

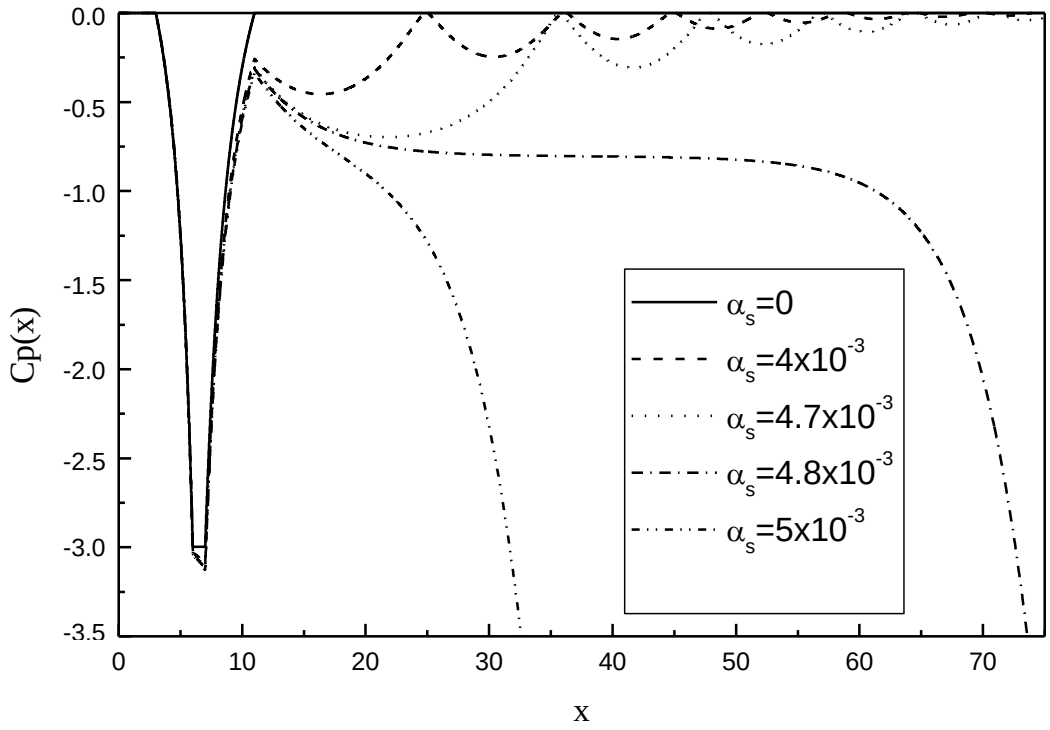

Figure 10. Fluid pressure coefficient for different upstream void fraction.

flow is studied. It can be observed that instability occurs for an upstream void fraction α_s>3,045.10^{-6}, which corresponds to a critical bubble radius r_c≈51. whereas, for our geometry (Figure 1), the same phenomenon occurs for α_s> 4,7.10^{-3}, with r_c≈4,3. This difference is due to the throat nozzle geometry. An other difference between these geometry's concerns the numerical implementation in the first case (Wang and Brennen, 1998) a variable space step is required, contrarily to the second case where a constant and relatively large space step is sufficient in the practice r_c correspond the flashing flow inception, which is illustrated by an instability of the parameters flow analytical expression for r_c is obtained by Wang and Brennen (1998), $R_c \approx (\sigma / 2\alpha_c)^{1/3}$, where α_c is the upstream void fraction at which flashing flow occurs.

The fluid velocity is illustrates in Figure 9. The presence of the bubble in the upstream flow results in the downstream fluctuations of the flow. With increase the upstream void fraction, the amplitude of this velocity fluctuations downstream increase and its frequency oscillation decrease. However, as a, increases to a critical value of the upstream void fraction, the flashing flow occurs, the velocity increases dramatically and the flow becomes unstable. Due to the Bernoulli effects, the fluid pressure coefficient varies inversely with the fluid velocity (Figure 10). Figure 11 illustrates the Bubble radius gradient in the flow for different upstream void fraction. Due to the inertial phenomena, the bubble radius gradient becomes a large value after the throat section of the venturi and a strongly dumping is also observed for the subsequent peaks. These peaks are reduced and amortized far further downstream flow.

CONCLUSION

A steady state equation set is considered for a bubbly two phase flow across a venturi. We have shown the effect of throat diameter and upstream void fraction on the characteristics parameters flow evolution. In the obtained result, we found that the upstream void fraction strongly affect the structure of the flow. Two different flow regimes are obtained: quasi-steady and quasi-unsteady regimes, where the transition between them is illustrated by a flashing flow inception. The latter phenomenon occurs at R_c≈ 4.3 which corresponds to α_s> 4.710^{-3}. This value is compared with the case of converging-diverging nozzle which indicates that the converging-diverging nozzle presents more stability than the venturi. This analytical result is numerically tested for a venturi. Also we have shown the inflexion point position and the corresponding bubble radius and void fraction.

Conflict of Interest

The authors have not declared any conflict of interest.

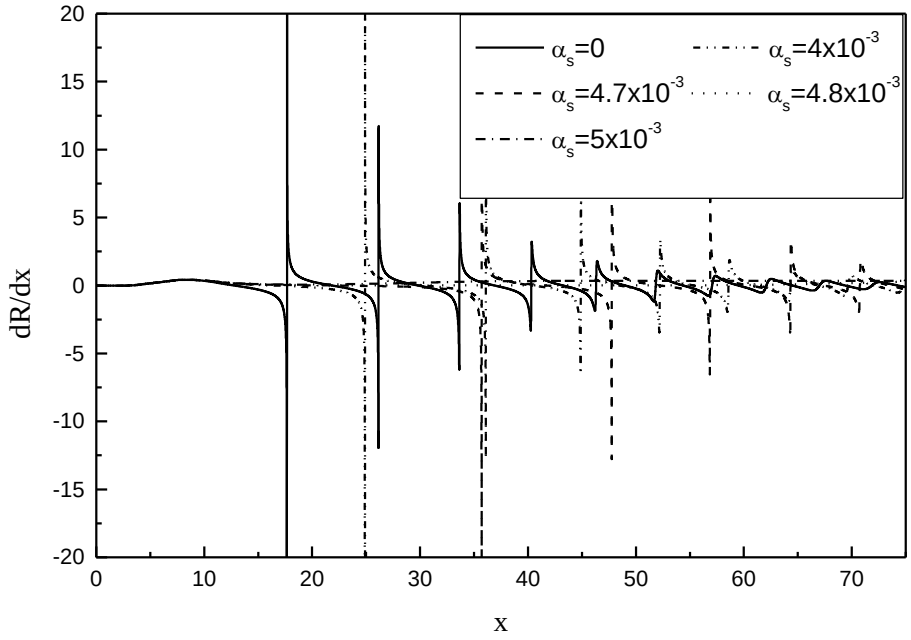

Figure 11. Bubble radius gradient for different upstream void fraction.

Nomenclature

A: dimensionless cross-sectional area of the Venturi, A^*/A_s^*, A*: cross-sectional area of the Venturi, A_s^*: upstream cross-sectional area of the Venturi, Cp: fluid pressure coefficient, $\left(p^* - p_s^*\right)/1/2\rho_L^* u_s^{*2}$, R: dimensionless bubble radius, R^*/R_s^*, R_c: dimensionless critical bubble radius at which flashing flow occurs, R_s^*: upstream bubble radius, Re: Reynolds number, $\rho_L^* u_s^* R_s^*/\mu_E^*$, S*: surface tension of the liquid, We: Weber number, $\rho_L^* u_s^{*2} R_s^*/S^*$, k: polytropic index for the gas inside the bubbles, p^*: fluid pressure, p_s^*:upstream pressure, p_v^*: vapor pressure, T: dimensionless time, $t^* u_s^*/R_s^*$, t*: time, u: dimensionless fluid velocity, u^*/u_s^*, u*: fluid velocity, u_s^*: upstream fluid velocity, V: volume of the bubble, $V = 4/3\pi R^3$, x: dimensionless Eulerian coordinate, x^*/R_s^*, x*: Eulerian coordinate.

Greek Letters

α: void fraction of the bubbly fluid, α_c: upstream void fraction at which flashing occurs, α_s: upstream void fraction, β: dimensionless radius of the Venturi throat, η: dimensionless bubble population per unit liquid volume, $\eta^* R_s^{*3}$, η^*: bubble population per unit liquid volume, γ: ratio of specific heats of the gas inside the bubbles, μ_E^*: effective dynamic viscosity of the liquid, ρ:dimensionless fluid density, ρ_L^*: density of the liquid, σ: cavitation number, $\left(p_s^* - p_v^*\right)/1/2\rho_L^* u_s^{*2}$.

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Scientific Research and Essays

Full Length Research Paper

Effect of pre-harvest foliar spray of calcium and potassium on fruit quality of Pear cv. Pathernakh

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Growing costs of fertilizers and increasing concern about ground water pollution has resulted indiscriminate or excessive soil fertilization problem that may be solved by more efficient fertilizer application technologies. The availability of new promising oriental pear or Japanese pear [*Pyrus pyrifolia* (Burm) Nakai] cultivar like 'Patharnakh', pave an opportunity for extending its cultivation under tarai region of Uttarakhand. Even though the cultivar 'Patharnakh' is good for tarai region but the quality is not much good to attract consumer acceptance. An experiment was conducted in 2013 to 2014 to study the effect of foliar spray of micronutrients on Pear. Fifteen year old pear trees were treated with three concentrations (1.0, 1.5 and 2.0%) of calcium and potassium nutrients *viz.,* calcium chloride, calcium nitrate, potassium sulphate and potassium nitrate; and water spray as a control at 30 days intervals starting from fruit set, that is, 20th March 2013, 20th April 2013 and 20th May, 2013. Each treatment was replicated thrice, in which one tree serving as a unit treatment. The experiment was conducted in Factorial Randomized Block Design. The observations were recorded on the basis of biochemical characters *viz.,* total soluble solids, acidity, ascorbic acid contents, total sugars, reducing sugar and non reducing sugar. Fruits treated with potassium nitrate at 1.5% showed the highest total soluble solids (11.72 ºBrix), total sugars (7.62%), reducing sugars (6.10%) and non reducing sugars (1.51%). However, titratable acidity (0.46 %), and ascorbic acid (6.42 mg/100 g) were found maximum with calcium chloride at 2.0% concentration. Therefore on the basis of economic point of view reducing the excessive cost of applied inputs by use of micronutrients can be an alternative to get quality produce. So, these treatments may be recommended for adaptation of Patharnakh pear in *tarai* region.

Key words: Pear, calcium and potassium spray, fruit quality.

INTRODUCTION

Pear (*Pyrus pyrifolia* Burm Nakai) is one of the important fruit of temperate region of the world. It can be grown in a wide range of climatic conditions and tolerate as low as -26°C temperature when dormant and as high as 45°C during growing period. A large number of pear cultivars require about 1200 h below 7°C during winter to meet their chilling requirements to flower and fruit satisfactorily. It belongs to the family Rosaceae, subfamily Pomoideae

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and genus *Pyrus,* having basic chromosome number 17 (2n=34). It ranks second, only next to apple in many aspects *viz.,* global importance, diversity of existence, acreage and production. About 1711.50 MT of pears are produced around the world, of which 20 MT are produced in India (FA0, 2014). The area under pear cultivation in the state of Uttrakhand is around 15.0 Thousand hectare, with annual production and productivity of 1.08 lack MT and 7.16 MT/ha, respectively (Anonymous, 2014). Macro and micronutrients play an important role in physiological characteristics and quality of pear. Amiri et al. (2008) claimed that foliar application of nutrients is more efficient to improve quality of pear, as foliar sprays can supply essential elements directly to the foliage and fruits. Improving marketable fruit with good quality has always been a challenge for scientists and pear growers. Growing costs of fertilizers and increasing concern about ground water pollution resulting from indiscriminate or excessive soil fertilization are problem that may be solved by more efficient fertilizer application technologies. The easiest way to maximize quality level by foliar spray is an alternative approach. Foliar application may be one technique which could possibly minimize the environmental hazard. Calcium plays an important role in maintaining the quality and storability of pear fruits. By applying calcium nutrition, the respiration rate was reduced, delaying ripening and there was increase in fruit firmness and the storage life was extended (Faust, 1979). Among various nutrients, foliar application of potassium is also considered to have profound effect on fruit quality that is colour, total soluble solids, acidity and vitamins contents (Bhargava et al., 1993). Gill et al. (2012) reported that under sub-tropical conditions of north-western India, pear cultivation is primarily focused to a single variety ‘Patharnakh’ due to its wider adaptability, low chilling requirement and higher yield potential. Therefore, the present study was carried out in order to study the effect of pre-harvest spray of calcium and potassium nutrients on fruit quality.

MATERIALS AND METHODS

The present investigation was carried out at Horticulture Research Centre Patharchatta, Govind Ballabh Pant University of Agiculture and Technolgy, Pantnagar (Uttarakhand) during the year 2013-2014. Fifteen year old pear trees were treated at three concentrations (1.0, 1.5 and 2.0%) of calcium and potassium nutrients sources from calcium chloride, calcium nitrate, potassium sulphate and potassium nitrate; and water as control at 30 days intervals’ starting from fruit set then on 20th March 2013, 20th April 2013 and 20th May, 2013. Each treatment was replicated thrice with one tree serving as a treatment unit. Control plants received only water sprays. Applications were made with foot sprayer using 10 L of water per plant. The experiment was conducted as Factorial Randomized Block Design (FRBD). Trees were maintained under uniform management practices and supplied with good quality tube well water for irrigation. The observations were recorded on the basis of various quality characters viz., total soluble solids, acidity, ascorbic acid contents, total sugars , reducing sugar and non reducing sugar. A random sample of 20 fruits from each replication was taken for physicochemical analysis. Pear fruits were harvested at their proper maturity on 15 July, 2013. Total soluble solid in fruits was recorded at room temperature using hand refractometer (Erma,Tokyo, Japan) and expressed in term of °Brix. A small amount of fruit pulp was taken in muslin cloth and crushed to obtain the juice of crushed pulp which was taken on the refractometer and the value was read against light. For acidity, fruit juice was titrated with 0.1 N NaOH and the results were expressed in terms of percentage of maleic acid as described by Rangana (1996). The Ascorbic acid was estimated by 2, 6-dichlorphenol-indophenol visual titration method as described by Ranggana (1996) and it was expressed in terms of mg per 100 g pulp. Sugars were estimated as per standard method Ranggana (1996). The observations were subjected to statistical analysis by using Factorial Randomized Block Design (FRBD) for various physicochemical attributes. Mean differences were tested by ‘F’ test at 5% level of significance (LOS). Critical difference (CD) at 5% level of significance was used for comparison among treatments.

RESULTS AND DISCUSSION

The data on total soluble solids presented in Table 1 reveal that all treatments were found to be significant except calcium chloride (T1) in terms of TSS. In the study, the highest total soluble solid content was recorded under potassium nitrate (11.72 °Brix), followed by potassium sulphate (11.11 °Brix) and calcium nitrate (11.03 °Brix) whereas, the minimum total soluble solid contents was recorded under control (10.42 °Brix). The mean TSS at various concentrations also differs significantly, the highest being recorded at 1.5% that is, 11.66 °Brix which is found statistically significant with C1 and C3. The interaction between treatment at their various concentrations (T × C) was found to be non significant. The possible reason of increase in TSS is adequate scope of nutrients to the plant, which hydrolyzed starch into sugar and helpful to increase the TSS of fruit. A higher increase in TSS content with foliar application of potassium is related with role of potassium in translocation of sugar from leaves to fruits, which results better quality fruits in term of total soluble solid. A marked influence in total soluble solid by these nutrients in current study is supported by Shirzadeh and Kazemi (2011) in Apple, Dhatt and Mahajan (2005) in Pear cv. Pathernakh, and Mahajan et al. (2008) in plum cv. Satluj Purple and Siddiqui et al. (1989) in ber cv. Umran.

The data presented in Table 1 manifests that spray of nutrients had a significant effect on titratable acidity over control. The highest mean titratable acidity was recorded under the calcium chloride (0.46%) followed by calcium nitrate (0.45%) and control (0.45%), while the minimum under potassium nitrate (0.41%). The mean acidity at various concentrations also differs significantly among themselves, the highest being recorded at 2.0% that is, 0.45% which is found statistically significant with C1 and C3. The interaction between treatments at various concentrations (T × C) was found to be non significant. The decrease in the acidity might be due to reduction in

Table 1. Effect of pre harvest spray of calcium and potassium on TSS (°Brix) and Titrable acidity of pear cv. Pathernakh.

Treatments	Fruit TSS (°Brix)				Titratable acidity (%)			
	Concentrations			Mean	Concentrations			Mean
	1.0% (C_1)	1.5 % (C_2)	2.0 % (C_3)		1.0 % (C_1)	1.5%(C_2)	2.0 % (C_3)	
Calcium Chloride (T_1)	10.63	10.91	11.07	10.87	0.47	0.45	0.44	0.46
Calcium Nitrate (T_2)	10.63	11.27	11.20	11.03	0.46	0.43	0.47	0.45
Potassium Sulphate (T_3)	11.21	11.41	10.70	11.11	0.43	0.42	0.45	0.43
Potassium Nitrate (T_4)	11.52	11.94	11.70	11.72	0.42	0.40	0.41	0.41
Mean	10.99	11.66	10.92		0.44	0.43	0.45	
	(T)	**(C)**	**(T × C)**		**(T)**	**(C)**	**(T × C)**	
C D at 5% or (p=0.005)	0.50	0.43	NS		0.013	0.011	NS	
	Control: 10.42 ± 0.33 (Mean ± SE)				**Control: 0.45 ± 0.011 (Mean ± SE)**			

the activities of enzyme by foliar application of these nutrients. Calcium and potassium nitrate being the source of nitrogen might have modified the vegetative growth, which in turn increase sugar metabolism and consequently decrease the acidity due to conversion of acid into sugar which resulted decrease in the acidity of fruits. The reduction in the acidity under potassium treatment might be owing to increased TSS of the fruits. Titratable acidity is directly related to the concentration of organic acids present in the fruit, which are an important parameter in maintaining the quality of fruits. These results also elucidate the finding of Gill et al. (2012) in pear cv. Pathernakh.

The significant differences were observed for mean values of ascorbic acid content for pear with the foliar application of calcium and potassium nutrients (Table 2). The range of variation for ascorbic acid content was from 6.15 to 6.42 mg/100 g of pulp. The maximum ascorbic acid (6.42 mg/100 g) was recorded with calcium chloride followed by 6.29 mg/100 g of pulp with calcium nitrate and 6.21 mg/100 g of pulp with potassium sulphate. At the same, minimum value (6.15 mg/100 g) for this trait was observed with water spray (control).The mean value of ascorbic acid content at various concentrations also differ significantly, the highest being recorded at 1.5%, that is, 6.39 mg/100 g of pulp (C_2) followed by 2.0%, that is, 6.35 mg/100 g of pulp (C_3), while the minimum ascorbic acid was recorded at 1.0% concentration, that is, 6.13 mg/100 g of pulp (C_1). The interaction between treatments at various concentrations (T × C) was further found to be significant, the highest (6.46 mg/100 g of pulp) being recorded with calcium chloride at 1.5% concentration ($T_1 \times C_2$) however, the lowest (5.96 mg/100 g) with potassium nitrate at 1.0% concentration ($T_4 \times C_1$). The increase in ascorbic acid content might be speculated due to increased activity of enzymes responsible for the synthesis of the ascorbic acid precursor and also the reduction in the rate of respiration by these chemicals. Bhat et al. (2009) reported the maximum ascorbic acid content with foliar application of calcium chloride (6.02 mg/100 g) and minimum under control (4.14 mg/100 g) in cherry cv. Makhmali, and suggested that the increases in ascorbic acid content might be attributed to higher synthesis of some metabolites and intermediate substances which promoted the synthesis of precursor of ascorbic acid and resulted the improvement in ascorbic acid content. However increase of ascorbic acid with foliar application of calcium and potassium nutrients are contradictory with the findings of Raese (1998) in Apple and Bhat et al. (2011) in pear cv. Bartlett.

Significant variation was observed with respect to data obtained from total sugar content in pear among all the nutrients applied over control (Table 2). The mean value of total sugar ranged from 7.15 to 7.62%. Among all the treatments under study, potassium nitrate recorded significantly highest total sugars (7.62%) followed by potassium sulphate (7.48%) and calcium nitrate (7.28%). The mean value of total sugar content at various concentration also differ significantly, the highest being recorded at 1.5%, that is, 7.63% (C_2) followed by 2.0%, that is, 7.38% (C_3) while the lowest was recorded at 1.0% concentration, that is, 7.15%. The interaction between treatment at various concentrations (T × C) was found also found to be significant however, the highest (7.76%) being recorded with potassium sulphate at 1.5% ($T_3 \times C_2$) and the lowest with calcium nitrate (7.07%) at 2.0% concentration ($T_2 \times C_3$). Singh et al. (2002) also reported that pre harvest sprays of nutrients have a marketed influence on increasing total sugar content of fruits over control, when applied at balloon bud stage and 15 days prior to maturity in peach cv. Flordasun. The effect of these nutrients on increase in total sugar contents could be attributed to the balance in nutrition status of the tree which advanced fruit maturity and ripening. The possible reason for increase in total sugar content may be due to hydrolysis of starch yielding mono and disaccharide, which owned a simplest form of sugar, and it could be one of the important reasons for the increase in total sugar content of fruits. Further higher levels of calcium do

Table 2. Effect of pre harvest spray of calcium and potassium on ascorbic acid content (mg/100 g) and total sugar (%) of pear cv. Pathernakh.

Treatments	Ascorbic acid (mg/100 g of pulp)				Total sugars (%)			
	Concentrations			Mean	Concentrations			Mean
	1.0% (C_1)	1.5% (C_2)	2.0% (C_3)		1.0% (C_1)	1.5% (C_2)	2.0% (C_3)	
Calcium Chloride (T_1)	6.36	6.46	6.43	6.42	7.21	7.53	7.36	7.36
Calcium Nitrate (T_2)	6.25	6.41	6.22	6.29	7.26	7.52	7.07	7.28
Potassium Sulphate (T_3)	5.94	6.36	6.34	6.21	7.23	7.76	7.45	7.48
Potassium Nitrate (T_4)	5.96	6.33	6.42	6.24	7.48	7.73	7.64	7.62
Mean	6.13	6.39	6.35		7.30	7.63	7.38	
CD at 5% or (p=0.005)	(T) 0.10	(C) 0.089	(T × C) 0.17		(T) 0.080	(C) 0.069	(T ×C) 0.14	
	Control: 6.15 ± 0.09 (Mean ± SE)				**Control: 7.15 ± 0.12 (Mean ± SE)**			

not showed improvement in total sugar (Table 2) reason being increased level of calcium in fruits which can retard ripening and senescence process resulting in slower hydrolysis of polysaccharides into monosaccharide and ultimately no further increase in sugar content of fruit. Our results are also in line with the finding of Raese (1998) in Apple.

A perusal of data presented in Table 3 revealed significance effects among all the treatments and concentrations in relation to reducing sugar in pear fruit. The highest mean reducing sugar was recorded under the potassium nitrate spray (6.10%) followed by potassium sulphate (6.06%) and calcium chloride (5.84%), while the minimum with control (5.78%). It also reveals from the Table 3 that mean reducing sugar at various concentrations differs significantly over control and themselves, the highest (6.15%) being recorded under 1.5% concentration (C_2), which was found significant with 1.0 and 2.0% concentration, while the lowest value of reducing sugar (5.86%) was recorded at 1.0% concentration (C_1). The interaction between treatments at various concentrations (T × C) was also found to be significant, the highest (6.24%) being recorded with potassium sulphate at 1.5% concentration ($T_3 \times C_2$) however, the lowest (5.70%) with calcium nitrate at 2.0% concentration ($T_2 \times C_3$). In agreement with our present findings Gill et al. (2005) reported that pre harvest spray of nutrients have a marked effect on non reducing sugar content of Kinnow Mandarin. Foliar spray of nutrients is helpful to increase the reducing sugar level, which could be due to translocation of carbohydrate as a result of maintenance of better assimilating power of laves over a longer period. An increase in reducing sugar with these nutrients may be due to the enhancement of photophosphorylation and dark reaction of photosynthesis by potassium and hence resulted in accumulation of more carbohydrates to the fruits, which results the better accessibility of nutrition for developing fruits and at long last increases the reducing sugar level of fruits. Similar observations have been reported by Bhat et al. (2012) in pear fruit cv. Bartlett, and Singh et al. (2002) in peach cv. Flordasun.

The data regarding the influence of various nutrients spray on non reducing sugars are presented in Table 3. It clearly indicates that various treatments have a significant effect on non reducing sugar content of fruit. The maximum mean value of non reducing sugar (1.51%) was observed with the potassium nitrate treatment (T_4), which was found significant with T_1, T_2 and T_3. The minimum non reducing sugars was reported under calcium chloride spray (T_1), that is, 1.38%, the interaction between treatment at various concentrations (T × C) was found to be non significant. The possible reason for increase in non reducing sugar content of fruits with the application of nutrients may be ascribed to hydrolysis of polysaccharides to simpler form, that is, mono and disaccharides and better transportation of nutrients to plant by potassium due to it important role in the transport of assimilates and nutrients to the plant from leaves to their place of utilization, which helps to increase availability of nutrition and conclusively better quality evolution in term of non reducing sugar content of fruits. These results corroborate the earlier records of Kumar et al. (1990) in grape cv. Delight, Bhat et al. (2009) in pear cv. Bartlett, Kaur and Dhillon (2006) in guava cv. Allahabad Safeda and Elham et al. (2007) in apricot cv. Canino. The results of present investigations revealed that pre harvest sprays of calcium and potassium nutrients at 1.5% were highly effective in improving total soluble solids, acidity, ascorbic acid, total sugars reducing sugars and non reducing sugar in Pathernakh pear.

Conclusion

Present studies clearly showed that different pre-harvest treatments of calcium and potassium at 1.5% had positive effect on quality of pear. Thus it can be concluded that potassium nitrate and calcium chloride

Table 3. Effect of pre harvest spray of calcium and potassium on reducing sugar (%) and non reducing sugar (%) of pear cv. Pathernakh.

Treatments	Reducing sugar (%)				Non reducing sugar (%)			
	Concentrations			Mean	Concentrations			Mean
	1.0% (C_1)	1.5% (C_2)	2.0% (C_3)		1.0% (C_1)	1.5% (C_2)	2.0% (C_3)	
Calcium Chloride (T_1)	5.75	6.15	6.04	5.98	1.46	1.38	1.32	1.38
Calcium Nitrate (T_2)	5.78	6.06	5.70	5.84	1.48	1.46	1.37	1.44
Potassium Sulphate (T_3)	5.91	6.24	6.03	6.06	1.32	1.51	1.42	1.42
Potassium Nitrate (T_4)	6.00	6.13	6.16	6.10	1.47	1.60	1.47	1.51
Mean	5.86	6.15	5.98		1.43	1.49	1.44	
	(T)	**(C)**	**(T × C)**		**(T)**	**(C)**	**(T ×C)**	
C D at 5% or (p=0.005)	0.043	0.037	0.074		0.076	0.066	NS	
	Control:5.78 ± 0.98 (Mean ± SE)				**Control:1.37 ± 0.046 (Mean ± SE)**			

improved the quality attributes of pear fruits cv. Pathernakh. Therefore in order to maintain the quality of the produce pre-harvest foliar spray of calcium and potassium with 1.5% can be recommended for the pear in *tarai* regions.

Conflict of Interest

The authors have not declared any conflict of interest.

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Scientific Research and Essays

Full Length Research Paper

Genetic divergence in some bivoltine silkworm (*Bombyx mori* L.) breeds

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Twenty-eight genotypes of bivoltine silkworm (*Bombyx mori* L.) were studied for genetic divergence using Mahalanobis D^2 statistic. Based on fifteen important metric traits, D^2 values were obtained and the genotypes were grouped into five clusters using Tocher's method. The clustering pattern indicated a mixed trend. The silkworm genotypes originating/evolved from different geographical regions fell in the same cluster. The inter-cluster distance ranged from 6142.45 to 19605.60. The genetic divergence was maximum between Clusters 5 and 2 followed by that between Cluster 3 and 2. Fifth age larval duration (19.56%), Total larval duration (15.93%), weight of mature silk gland (16.73%), Single cocoon weight (11.29%), Cocoon yield/10,000 larvae by No. (22.78%) and Denier (13.11%) contributed maximum towards the total genetic divergence. The results reveal that while identifying parents for hybridization programme, genetic distances and not the geographic diversity are to be considered. The choice of characters is also important while planning the cross breeding programmes.

Key words: Cluster, genetic divergence, heterosis, silkworm.

INTRODUCTION

In order to synthesize high yielding silkworm hybrids it is important to have a collection of varied gene pool of silkworm races, because variability is the basic requirement for the genetic improvement of any crop. Therefore, to create new reservoirs of genetic variability cross breeding strategies have been extensively used as a means of harnessing heterosis in the bivoltine silkworms (Narayanswamy et al., 2002). One of the challenges in the silkworm breeding is the selection of suitable parental lines with which to develop heterotic combinations. Determining genetic divergence among the available lines has been seen to facilitate this task. Genetic diversity is critical to success in any crop breeding and it provides information about the quantum

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Table 1. Representation of genotypes in different clusters.

Cluster No.	No. of genotypes	Genotypes
1	24	J_{122}, NCD. Sannish, JA_1, NB_{18}, CSGRC-5, Jam_{21}, Jam_{18}, CSR_2, JD_6, YS_3, A, SRC, New race, $Pure_{81}$, J_2M, Sheiki II, Pampore-5, Meigitsu, 14M, CSR_4, NB_4D_2, Belkokona II and SPJ_2.
2	1	SH_6
3	1	B_{38}
4	1	NJ_3
5	1	JBEL

of genetic divergence and serves a platform for specific breeding objectives. Genetic diversity is a particular concern because greater genetic uniformity in silkworm can increase vulnerability to pests and diseases. Hence, maintenance of genetic diversity is a fundamental component in long-term management strategies for genetic improvement of silkworm (Bindroo and Moorthy, 2014).

Crossing of genetically diverse parents helps in the recombination of genes from diverse resources (Siddiqui et al., 1992) producing high heterotic effects and more variability in segregating generations. Hence selection of genetically pure and divergent parental strains is critical to the success of a hybridization programme in silkworm (Nagaraju and Goldsmith, 2002). Since a great deal of variability exists among various silkworm breeds it is imperative for a breeder to have a knowledge on the genetic distances and the nature and magnitude of genetic diversity among the available silkworm breeds as it is a pre-requisite for choosing parents for hybrids which in turn results in the elevation of quantitative and qualitative aspects of cocoon production (Sen et al., 1996). Various statistical tools are available to measure quantitatively the genetic divergence existing among populations. Mahalanobis (1936) developed a statistical model to study the genetic divergence among populations. This technique has been widely copied by various researchers over the years in choosing parents for hybridization in crop plants (Gupta et al., 1991; Vijayan et al., 1999). Of late, this technique has also been used to ascertain the magnitude of genetic divergence in silkworm, *Bombyx mori* L. (Farooq et al., 2005). Since all the silkworm genotypes have not been ascertained, the investigation was, therefore, undertaken to study the genetic divergence among some more silkworm *B. mori* L. genotypes.

MATERIALS AND METHODS

The present investigation was carried out at the Division of Sericulture, SKUAST (K), Mirgund during 2007-2009.The material for the present investigation comprised 28 bivoltine silkworm lines viz., New Race, $Pure_{81}$, Pampore-5, J-122, Meigitsu, JA_1, 14M, SPJ-2, J_2M, B_{38}, CSGRC-5, Belkokona II, Sheiki II, Sannish, A, Jam $_{18}$, Jam $_{21}$, JD_6, YS_3, NJ_3, NCD, NB_{18}, NB_4D_2, CSR_2, CSR_4, SH_6, SRC, JBEL, of different origin obtained from the germplasm bank maintained at Division of Sericulture, Mirgund, germplasm bank maintained at SKUAST (J), Udaiwala, Jammu and germplasm bank maintained at Central Sericultural Germplasm Resource Centre, Hosur. The silkworm genotypes were reared consecutively two times in a Completely Randomised Block Design with three replications for each treatment; each replication comprised 250 worms after 3rd moult. The rearing were conducted following methods suggested by Dar and Singh (1998).

The data pertaining to the following parameters were recorded and subjected to analysis of variance: 5th age larval duration, total larval duration, weight of mature larvae, weight of mature silk gland, single cocoon weight, single shell weight, shell ratio, cocoon yield/10,000 larvae (by number and weight), pupation rate, filament length, denier, raw silk percentage, fecundity and hatching percentage. The data was put to Mahalanobis D^2 analysis for assessing the genetic divergence among the populations and the contribution of individual characters towards divergence. Clustering was done by Tocher's method as described by Singh and Chaudhary (1977). Relative contribution of each character towards genetic divergence and average intra and inter-cluster distances were also estimated as described by Singh and Chaudhary (1977). Significance of differences among genotypes was tested using Wilk's criterion.

RESULTS AND DISCUSSION

The silkworm genotypes, their place of origin and source are presented in Table 1. On the basis of D^2 values, which were computed for each pair of genotypes, the twenty eight genotypes were grouped into 5 clusters (Table 1). The first cluster included twenty four genotypes viz., J_{122}, NCD, Sannish, JA_1, NB_{18}, CSGRC-5, Jam $_{21}$, Jam_{18}, CSR_2, JD_6, YS_3, A, SRC, New race, Pure 81, J_2M, Sheiki II, Pampore-5, Meigitsu, 14M, CSR_4, NB_4D_2, Belkokona II and SPJ_2. The remaining four silkworm genotypes viz., SH_6, B_{38}, NJ_3 and JBEL constituted second, third, fourth and fifth cluster, respectively.

The cluster mean for fifteen economic traits is presented in Table 2. There was a wide range of variation in some of the characters. The 5th age larval duration ranged from 165.26 h in Cluster 1 to 177.35 h in Cluster 5 whereas, minimum total larval duration was found in Cluster 4 (636.40 h) and maximum in Cluster 2 (653.88 h). The maximum weight of mature larvae was found in Cluster 2 (49.43 g $larva^{-10}$) and minimum in Cluster 3

Table 2. Cluster means for different characters.

Cluster No.	5th age larval duration (hr.)	Total larval duration (hr.)	Weight of mature larvae (g.larva-10)	Weight of silk gland (g.)	Single cocoon weight (g.)	Single shell weight (g.)	Shell ratio (%)	Cocoon yield/ 10,000 larvae by no.	Cocoon yield/ 10,000 larvae by wt. (kg.)	Pupation rate (%)	Filament length (m.)	Denier	Raw silk (%)	Fecundity	Hatching (%)
1	165.26	645.25	43.74	1.50	1.88	0.34	18.29	8610	16.17	84.93	827	2.82	13.61	564	93.38
2	173.88	653.88	49.43	1.53	2.19	0.37	17.05	9149	20.03	86.29	870	2.68	13.43	609	97.65
3	165.28	645.28	41.17	1.99	1.76	0.30	17.04	6872	12.09	85.62	883	2.78	12.04	608	93.99
4	166.40	636.40	41.77	1.25	1.65	0.31	18.54	8860	14.65	83.96	627	3.32	14.38	580	90.05
5	177.35	641.35	44.63	1.39	1.81	0.32	17.68	8338	15.09	83.97	893	2.27	12.68	559	95.64

(41.77 g larva^{-10}). Weight of mature silk gland was minimum (1.25 g) in Cluster 4 and maximum (1.99 g) in Cluster 3. The maximum single cocoon weight (2.19 g) and maximum single shell weight (0.37 g) were revealed by Cluster 2 whereas minimum single cocoon weight (1.65 g) and minimum shell weight (0.30 g) were recorded in Clusters 4 and 3, respectively. However, Cluster 4 exhibited highest shell ratio (18.54%) and Cluster 3 the lowest (17.04 %). The maximum cocoon yield/10,000 larvae by number (9149) and by weight (20.03 kg) were found in Cluster 2 whereas minimum cocoon yield/ 10,000 larvae by no. (6872) and by wt. (12.09 kg) were found in Cluster 3. Pupation rate ranged from 83.96 % in Cluster 4 to 86.29% in Cluster 2. Cluster 5 revealed longest filament (893.67 m) whereas, Cluster 4 exhibited shortest filament (627.33 m). Maximum denier (3.32) was found in Cluster 4 and minimum denier (2.27) in Cluster 5. Raw silk percentage ranged from 12.04 in Cluster 3 to 14.38 in Cluster 4. The highest fecundity (609) and hatching percentage (97.65%) was revealed by Cluster 2 whereas minimum fecundity (559.33) was found in Cluster 5 and minimum hatching percentage (90.05%) was found in Cluster 4. Cluster 2 comprising of SH_6 only had the highest values for nine characters viz., Total larval duration, weight of mature larvae, single cocoon weight, single shell weight, cocoon yield/10,000 larvae by number and by weight, pupation rate, fecundity and hatching percentage. However, Cluster 3 was poorest in six characters viz., weight of mature larvae, single shell weight, shell ratio, cocoon yield/10,000 larvae by number and by weight and raw silk percentage and Cluster 4 was poorest in other six characters viz., total larval duration, weight of mature silk gland, single cocoon weight, pupation rate filament length and hatching percentage.

The average intra- and inter-cluster distances are presented in Table 3 and Figure 1. The inter-cluster distances ranged from 6142.45 to 19605.60. The genetic divergence was maximum between Clusters 5 and 2 (19605.60) followed by that between Clusters 3 and 2 (17109.14), Clusters 5 and 3 (16227.71), Clusters 5 and 1 (14675.27), Clusters 4 and 3 (14343.42), Clusters 4 and 2 (13484.62), Clusters 4 and 1 (8281.53), Clusters 2 and 1 (8062.63), Clusters 3 and 1 (7424.25) and between Clusters 5 and 4 (6142.45). The inter-cluster distances were greater than the intra- cluster distance indicating the presence of high degree of genetic divergence. While analyzing the contribution of various characters towards the expression of genetic divergence (Table 4), it was found that 5th age larval duration (19.56%), total larval duration (15.93%), weight of mature silk gland (16.73%), single cocoon weight (11.29%), cocoon yield/10,000 larvae by number (22.78%) and denier (13.11%) contributed maximum towards the total genetic divergence. These characters accounted for 99.40% of the total genetic divergence in the material. The least contribution to genetic divergence was made by weight of mature larvae, pupation rate and filament length (0.20% each). The characters single shell weight, shell ratio, cocoon yield/10,000 larvae by weight, raw silk percentage, fecundity and hatching percentage did not contribute to genetic divergence in the present set of materials.

The twenty-eight silkworm genotypes formed five distinct clusters. All the silkworm genotypes originating from Japan fell in Cluster 1 except B_{38} which constituted a single solitary cluster. Among seven genotypes evolved in Mysore five viz., NCD, NB_{18}, CSR_2, CSR_4 and NB_4D_2 fell in Cluster 1 and one NJ_3 formed a separate cluster. The three silkworm genotypes (Sannish, Sheiki II and Belkokona II) originating from Russia and two silkworm genotypes (Jam $_{21}$, Jam $_{18}$) evolved in Jammu also fell in Cluster 1. Out of two races (SRC and JBEL) evolved in West Bengal one, SRC fell in Cluster 1 and JBEL formed a single separate cluster. Cluster 1 also included CSGRC-5 evolved at CSGRC, Hosur, JD_6 and YS_3 evolved in Dehradun and A, originated in Poland. However, SH_6, a genotype evolved in Dehradun formed a separate solitary cluster.

The clustering pattern indicated a mixed trend.

Table 3. Average inter- and intra-cluster distances.

Cluster	1	2	3	4	5
1	(4546.91)	8062.63	7424.25	8281.53	14675.27
2		(0.00)	17109.14	13484.62	19605.60
3			(0.00)	14343.42	16227.71
4				(0.00)	6142.45
5					(0.00)

Figures in parenthesis indicate intra-cluster distance.

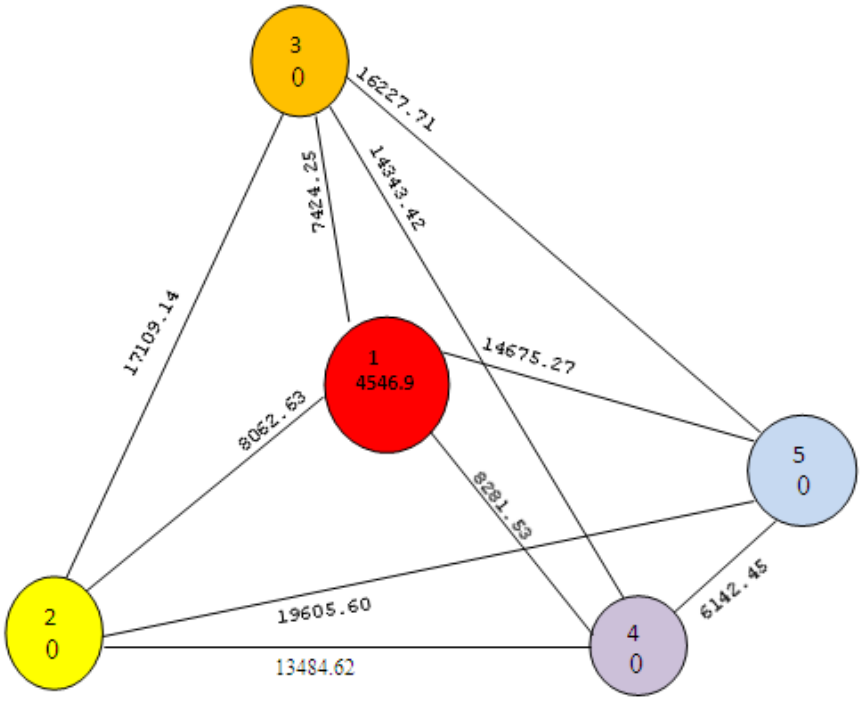

Figure 1. Mahalanobis Euclidean Distances (Not to the scale).

The silkworm genotypes originating/evolved from different geographical regions viz., Japan, Russia, Mysore, Hosur, Jammu, Dehradun, Poland, Pampore and West Bengal, fell in the same cluster. It has been reported by Farooq et al., 2005, that silkworm genotypes originating from different geographical regions fell in one cluster, while those originating from a single geographic region fell in different clusters. Ahmad and Borah, (1999) reported that the relative contribution of different genotypes into different clusters at times reveals no parallelism between genetic diversity and geographical origin. The populations overtime differentiate due to human selection and genetic drift and get adapted to specific agroclimatic environments leading to divergence. Zanatta et al., (2009) also reported that the silkworm strains of same origin did not group together demonstrating they can have different biological and developmental performance. Nehzad et al., (2010) also revealed the inclusion of genotypes of the same origin in different clusters.

The results reveal that while identifying parents for hybridization programme, genetic distances and not the geographic diversity are to be considered. The inter-cluster distances in the present investigation are high showing considerable degree of divergence among various clusters. So the parents for hybridization and future breeding programme can be selected from among the divergent groups. Among the fifteen quantitative characters studied, six characters viz., 5^{th} age larval duration, total larval duration, weight of mature silk gland, single cocoon weight, cocoon yield/10,000 larvae by number and denier contributed about 99.40% towards the total genetic divergence. The results also indicate that the choice of the characters is also important as pointed out

Table 4. Contribution of each character towards genetic divergence.

Character	5th age larval duration (hr.)	Total larval duration (hr.)	Weight of mature larvae (g. larva^{-10})	Weight of silk gland (g.)	Single cocoon weight (g.)	Single shell weight (g.)	Shell ratio (%)	Cocoon yield/ 10,000 larvae by no.	Cocoon yield/ 10,000 larvae by wt. (kg.)	Pupation rate (%)	Filament length (m.)	Denier	Raw silk (%)	Fecundity	Hatching (%)	Total
No. of times appearing first in ranking	97	79	1	83	56	0	0	113	0	1	1	65	0	0	0	496
Percentage contribution	19.56	15.93	0.20	16.73	11.29	0.00	0.00	22.78	0.00	0.20	0.20	13.11	0.00	0.00	0.00	100

by Farooq et al. (2004). It could be conceived that these yield contributing characters must be taken into consideration while planning the cross breeding programmes.

Conflict of Interest

The authors have not declared any conflict of interest.

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Scientific Research and Essays

Full Length Research Paper

Larval stage of trematodes obtained from brackish water snails in the central and east coast of the gulf of Thailand

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Brackish water snail is one of the important intermediate hosts of trematode infections. This kind of snail along the Gulf of Thailand were examined for cercariae, the larval stage of trematodes. The aim of this study was to investigate cercarial infections in brackish water snails in the central and eastern side of the Gulf of Thailand. Snails were collected from 45 sampling sites (20 central areas, 25 eastern areas), between August 2013 and February 2014. A total of 8,532 snails were classified into 12 families, 23 genera and 32 species. Cercarial infections were investigated using shedding and crushing methods. The infection rate was 0.27% (23/8,532). The cercariae were categorized into 5 types and 8 species. The first type, Parapleurophocercous cercariae, consisted of *Haplorchis taichui*, *Heterophyes cercaria* I, and *Metorchis intermedius*. The second type, Xiphidiocercariae, consisted of *Ascorhytis charadriformis.* The third type, Furcocercous cercariae, consisted of *Apharyngostrigea pipientis.* The forth type, Cotylomicrocercous cercariae, consisted of *Coitocaecum anaspidis*. The fifth type, Echinostome cercariae, consisted of *Hypoderaeum conoideam* and *Himasthla interrupta*. Seven species of snails were found with trematode infections, comprising of *Assiminea brevicula*, *Cerithidea cingulata*, *Cerithidea alata*, *Cerithidea djadjariensis*, *Cerithidea quadrata*, *Littorinopsis intermedia,* and *Sermyla riqueti*.

Key words: Cercaria, brackish water snails, trematode, the Gulf of Thailand.

INTRODUCTION

Thailand is situated between the Gulf of Siam and the Andaman Sea. Along the coasts line sand beaches, rock beaches, mangrove forests and river tributaries, providing abundant habitats where various aquatic fauna resided, including molluscs. It is well known that both gastropods and bivalves could be the first intermediate hosts of human and animal trematodes. The mollusc-transmitted diseases are very important for the veterinary and public health. Trematodes need two or three hosts to complete their life cycles. Trematode eggs hatch in the water. At

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the larval stage, they can swim to find the first intermediate hosts, such as freshwater snails, brackish water snails, and terrestrial snails. In the snails, miracidium develops into sporocyst to redia and finally to cercaria. Cercaria leave the snails and head for the second intermediate host, e.g. fish or amphibian. Then they develop into metacercaria. Animals and humans can be infected by eating metacercaria or by the penetration of cercaria. The study of brackish water snails as intermediate hosts of trematode was reported. *Pirenella conica* (Gastropoda: Potamididae), a small sea snail along the coasts of Sinai and Israel were infected with trematode of the family Heterophyidae, Echinostomatidae, Microphallidae, Notocotylidae, Haploporidae, Haplosplanchnidae, Cyathocotylidae and Strigeidae (Taraschewski and Paperna, 1981). *Cerithidea cingulata* of Kuwait Bay found with echinostome cercaria infection was reported as new cercaria species by Salam and Sreelatha (1999). Moreover, the snail, *C. cingulata* of Kuwait Bay were infected with 12 species of trematodes. They belong to the family Cyathocotylidae, Echinostomatidae, Haplosplanchnidae, Heterophidae, Microphallidae, Philophthalmidae, Plagiorchiidae and Schistosomatidae (Al-Kandari et al., 2000). *Zeacumantus subcarinatus* (Prosobranchia: Batillaridae), the southern creeper, was reported as a new echinostome cercaria (Marorelli et al., 2006).

In Thailand, mud whelks in the family Potamididae (*Cerithidea cingulata, C. djadijarensis* and *C. charbonnieri*) could be intermediate host of 3 groups of cercariae. They were separated by morphological characters. The first group was found with a cystogenous gland but with no eye spots and no collar spines. The second group was found with eye spots, 3-4 penetration glands, and a finfold tail. The third group was still undetermined (Sri-aroon et al., 2005). Those cercariae are not known species. And those surveys were limited to only some regions of Thailand (Sri-aroon et al., 2004, 2005, 2010). Therefore, the aim of this study was to investigate and identify trematode infections in brackish water snails in Central and Eastern Thailand. The knowledge of trematode fauna can provide more understanding for veterinary and public health control.

MATERIALS AND METHODS

Sampling sites and collection of snails

Snails were collected from 45 sampling sites (20 central areas, 25 east areas), between August 2013 and February 2014. The counts per unit of time method was used for snail sampling. Five collectors picked the snails by hand and scoop for 10 minutes each station. The sampling sites were mangrove forest, drainage, canal and tributary of river in Trat, Chanthaburi, Rayong, Chon Buri, Chachoengsao, Bangkok, Samut Pakran, Samut Sakhon and Samut Songkhram provinces of Thailand (Figure 1). The collected snails were transferred to the Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Thailand. Those snails were identified by shell morphology (Brandt, 1974; Upatham et al., 1983).

Examination for trematode infections

Trematode infections were investigated by using snail shedding and crushing methods. Cercariae were collected in dechlorinated water and examined under stereo-microscope and scored the infection rates.

Study of cercarial morphology

Cercarial morphology and anatomy were examined by stained cercariae. They were stained with 0.5% neutral red and identified under light microscope. The cercariae were fixed in 10% formalin, then measured and averaged from 10 specimens. The stained specimens were drawn using *camera lucida*. Scanning electron images of cercariae were taken by Camscan MX 2000 scanning electron microscope (UK). The cercariae were processed by fixed in 2.5% glutaraldehyde phosphate buffer at 4°C for at least 2 h and post-fixed in 1% osmium tetroxide at 4°C for 1 h, dehydrated through a graded series of acetone, then they were air dried. The specimens adhere to carbon stub, were coated with gold-palladium in an ion-sputtering apparatus (Polaron CPD 7501, UK), then examined under SEM. The species of trematodes were identified by their morphology and anatomy following Yamaguti (1975) and Ito (1980).

RESULTS

Snail samples and Cercarial infections

A total of 8,532 brackish water snails were collected from estuaries and mangrove forests along the coast of Central and Eastern parts of Thailand. They were categorized into 32 species of 12 families: Neritidae, Littorinidae, Stenothyridae, Iravadiidae, Assimineidae, Thiaridae, Muricidae, Potamididae, Ellobiiidae, Naticidae, Viviparidae and Cerithiidae. Twenty-three genera and thirty-two species were categorized (Table 1). Seven species were found as intermediate hosts of trematodes, which were *Assiminea brevicula* (Assimineidae), *Cerithidea cingulata*, *C. alata*, *C. djadjariensis*, *C. quadrata* (Potamididae), *Littorinopsis intermedia* (Littorinidae) and *Sermyla riqueti* (Thiaridae)(Figure 2). The infection rates were 0.14% (1/691), 0.09% (1/1,068), 0.13% (1/763), 0.32% (2/623), 4.20% (5/119), 0.61% (7/1,154) and 0.42% (6/1,437), respectively (Table 2). The obtained cercariae were classified into 5 types and 8 species. The first type was Parapleurophocercous cercariae *(Metorchis intermedius, Heterophyes cercaria* I*, Haplorchis taichui*); the second type was Xiphidiocercariae (*Ascorhytis charadriformis*); the third type was Furcocercous cercariae (*Apharyngostrigea pipientis*); the forth type was Cotylogmicrocercous cercariae (*Coitocaecum anaspidis*); and, the fifth type was Echinostome cercariae (*Hypoderaeum conoideam* and *Himasthla interrupta*).

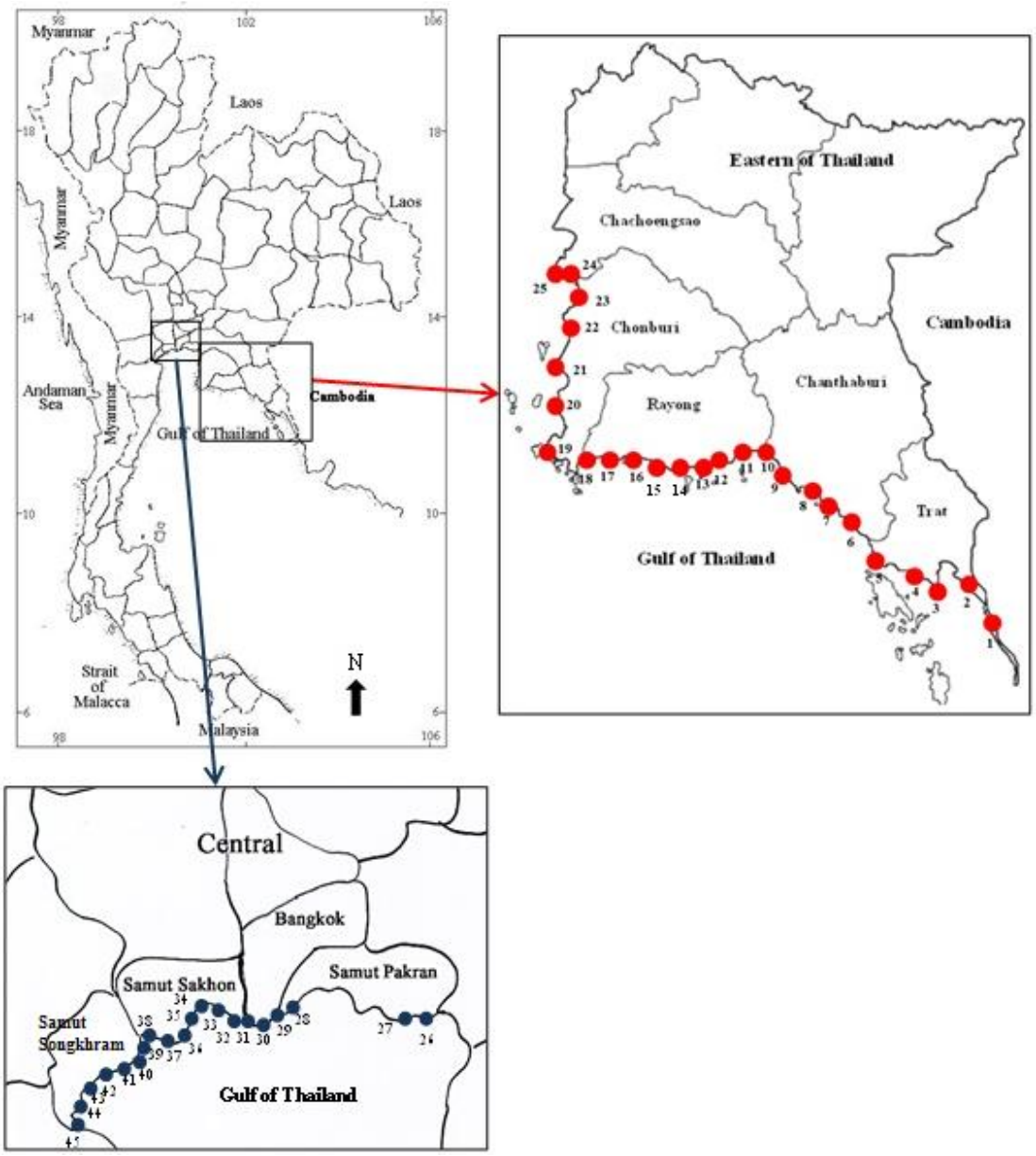

Figure 1. Maps of Thailand showing 5 provinces on the east coast: locations 1-25 (Trat, Chanthaburi, Rayong, Chonburi, and, Chachoengsao); and, 4 provinces in the middle of the Gulf: locations 26-45 (Samut Prakarn, Bangkok, Samut Sakhon, and, Samut Songkhram).

Morphology of cercariae

Characteristics of cercariae were described from fixed cercariae and living cercariae. Size of cercariae were measured for identification of cercarial species.

Type 1. Parapleurophocercous cercariae

Metorchis intermedius Heinemann, 1937

The body was oval in shape and yellowish brown in color. Four rows of spines were found around an oral sucker. There was a long prepharynx. Esophagus and ceca were not found. One pair of eye spots were apparently without lens. Fourteen penetration glands formed a compact median groups of 3:4:4:3 each. The ventral sucker was a prevesicular cell. The excretory vesicle was round, and lined with epithelia. Tail tubule median opened at a distance from the base of tail. The dorsal finfold began at the posterior end of basal swelling and passed over to the ventral finfold which reached the middle of the tail. The cercariae were produced within the redia (Figure 3).

The following figures are size ranges and average sizes

Table 1. Brackish water snalis were collected between August 2013 and February 2014 from the Central and Eastern Coasts of the Gulf of Thailand.

Family	Genus / Species	Province* / no. of collected snails (no. of infected snails)									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
Assimineidae	1. *Assiminea brevicula*	30	0	29	342	0	0	87(1)	53	150	**691**
Cerithiidae	2.*Clypeomorus moniliferus*	0	0	3	0		0	0	0	0	**3**
Ellobiidae	3.*Cassidula aurisfelis*	16	107	96	44	0	0	6	0	19	**288**
	4. *Cassidula mustelina*	1	1	12	9	0	0	0	3	0	**26**
	5.*Cassidula multiplicata*	0	0	1	1	0	0	0	0	3	**5**
	6. *Ellobium aurisjudae*	0	15	4	24	0	0	2	0	1	**46**
	7. *Laemodonta punctigera*	0	1	0	10	0	0	9	0	26	**46**
	8. *Laemodonta siamensis*	0	0	0	5	0	0	0	0	0	**5**
	9. *Micromelampus siamensis*	0	0	1	0	0	0	0	88	0	**89**
	10. *Melampus nucleolus*	0	0	0	0	0	0	0	0	2	**2**
Iravadiidae	11.*Fairbankia cochinchinensis*	0	57	0	0	0	0	0	0	0	**57**
Littorinidae	12. *Littorinopsis intermedia*	0	1	116	13	0	0	58(3)	240	726(4)	**1154**
	13. *Littorinopsis scabra*	43	29	42	1	0	0	0	5	0	**120**
	14. *Littorinopsis melanostoma*	0	0	2	40	0	0	0	1	3	**46**
Muricidae	15.*Chicoreus capucinus*	10	5	22	0	0	0	0	0	0	**37**
Naticidae	16. *Euspira* sp.	0	0	0	4	0	0	0	23	0	**27**
Neritidae	17. *Clithon peguensis*	339	103	311	181	0	0	0	0	0	**934**
	18. *Dostia violacea*	0	6	3	24	0	2	0	3	58	**96**
	19. *Neritodryas cornea*	0	0	0	0	0	0	0	0	22	**22**
Potamididae	20. *Cerithidea alata*	66(1)	51	40	137	0	0	0	0	0	**294**
	21. *Cerithidea cingulata*	134	125	153	188	0	0	446(1)	5	17	**1068**
	22.*Cerithidea djadjariensis*	107	129	345(2)	42	0	0	0	0	0	**623**
	23.*Cerithidea quadrata*	34	20(1)	35(3)	16(1)	0	0	0	0	0	**105**
	24.*Cerithidea obtusa*	0	0	0	5	0	0	0	0	0	**5**
	25. *Telescopium telescopium*	0	2	0	0	0	0	0	0	0	**2**
	26. *Terebralia palustris*	12	0	1	0	0	0	0	0	0	**13**
	27. *Faunus ater*	0	0	34	0	0	0	0	0	0	**34**
Stenothyridae	28. *Stenothyra* sp.	0	0	0	1	0	188	0	4	0	**193**

Table 1. Contd.

	29. *Melanoides tuberculata*	0	7	0	0	7	0	0	9	3	**26**
Thiaridae	30. *Sermyla riqueti*	0	6	130[(4)]	196[(1)]	0	14	37[(1)]	417	118	**918**
	31. *Tarebia granifera*	0	0	2	0	904	0	0	341	303	**1550**
Viviparidae	32. *Filopaludina martensi*	6	0	0	0	1	0	0	0	0	**7**
	Total	**798**	**665**	**1382**	**1283**	**912**	**204**	**645**	**1192**	**1451**	**8,532**

* Eastern Provinces: 1. Trat 2. Chanthaburi 3. Rayong 4. Chon Buri 5. Chachoengsao Central Provinces: 6. Samut Prakarn 7. Bangkok 8. Samut Sakhon 9. Samut Songkhram.

of 10 cercariae:

Body: 88-103 µm (av. 93 µm) x 180-183 µm (av. 180 µm)
Tail: 25-28 µm (av. 27 µm) x 338-430 µm (av. 373 µm)
Eye spot: 10-20 µm (av. 15 µm) x 8-15 µm (av. 10 µm)
Oral sucker: 28-40 µm (av. 35 µm) x 28-43 µm (av. 35 µm)
Pharynx: 15-23 µm (av. 19 µm) x 10-13 µm (av. 11 µm)
Excretory bladder: 70-88 µm (av. 78 µm) x 45-55 µm (av. 50 µm)
Lateral finfold: 25-28 µm (av. 27 µm) x 175-180 µm (av. 178 µm)
Dorso-ventral finfold : 15-18 µm (av. 16 µm) x 158-163 µm (av. 160 µm)

Heterophyes cercaria I Martin, 1959

The body was oval in shape and yellowish brown in color. The body surface was covered with tiny barbed and sensory hair. Pre-pharynx was found. Pharynx was small and round in shape. Eye spots were round and triangular in shape. Seven pairs of penetration glands were in two longitudinal rows. Their ducts were in 2 bundles. Acetabulum was not observed. Excretory vesicle was thick wall and V-shape. Tail was tubule with finfolds. The characteristics of finfolds were shown; the dorsal fin arose from the tail base, extended to the whole length of the tail, and continued with the ventral fin to the tail tip. The excretory duct opened at the tail tip. The cercariae were produced within the rediae (Figure 4). The following figures are size ranges and average sizes of 10 cercariae:

Body: 98-130 µm (av. 114 µm) x 163-195 µm (av. 179 µm)
Tail: 40-45 µm (av. 43 µm) x 523-580 µm (av. 551 µm)
Eye spot: 10-15 µm (av. 12 µm) x 8-10 µm (av. 9 µm)
Oral sucker: 33-38 µm (av. 35 µm) x 23-33 µm (av. 28 µm)
Pharynx: 13-15 µm (av. 14 µm) x 15-18 µm (av. 16 µm)
Excretory bladder: 23-25 µm (av. 24 µm) x 65-70 µm (av. 68 µm)
Dorso-ventral finfold: 30-40 µm (av. 35 µm) x 250-280 µm (av. 265 µm)

Haplorchis taichui (Nishigori, 1924) Witenberg, 1930

The body was oval in shape and orange or yellow in color. Spines and sensory hairs covered the whole body. An oral sucker was situated in the head region. The mouth aperture had transverse rows of spines. One pair of eye spots and a pharynx were present. Seven pairs of penetration glands extended from the pharynx to the end of the body. There were two longitudinal rows of genital primodia. The excretory bladder had a round shape and was composed of fine pigments. A long tail was attached to the dorsal end of the body, with lateral finfolds and a dorso-ventral finfold.
The cercariae were produced within the rediae (Figure 5). The following are size ranges and average sizes of 10 cercariae:

Body:75-100 µm (av. 83 µm) x 80-113 µm (av. 100 µm)
Tail: 20-35 µm (av. 28 µm) x 325-380 µm (av. 357 µm)
Eye spot: 8-13 µm (av. 10 µm) x 5-10 µm (av. 8 µm)
Oral sucker: 38-45 µm (av. 41 µm) x 35-45 µm (av. 40 µm)
Ventral sucker: 10-13 µm (av. 12 µm) x 8 -13 µm (av. 12 µm)
Pharynx: 10-15 µm (av. 11 µm) x 10-13 µm (av. 11 µm)
Excretory bladder: 48-55 µm (av. 52 µm) x 13-23 µm (av. 16 µm)

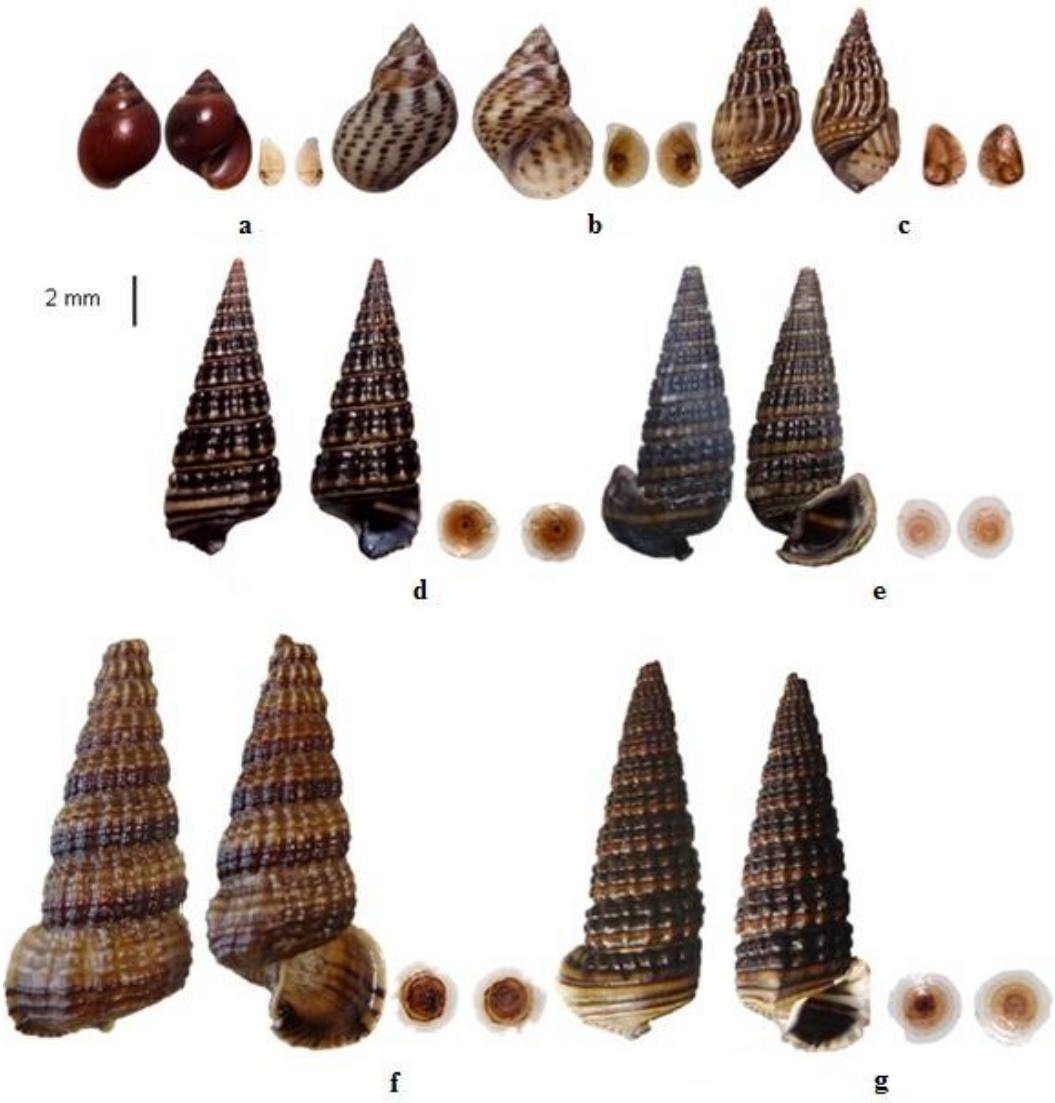

Figure 2. Seven species of brackish water snails of the Central and the Eastern Coasts along the Gulf of Thailand found with cercarial infections (a) *Assiminea brevicula* (b). *Littorinopsis intermedia* (c). *Sermyla riqueti* (d). *Cerithidea cingulata* (e). *C. alata* (f). *C. quadrata* (g). *C. djadjariensis*.

Lateral finfold: 10-18 µm (av. 12 µm) x 120-158 µm (av. 134 µm)
Dorso-ventral finfold: 8-18 µm (av. 13 µm) x 145-238 µm (av. 194 µm)

Type 2. Furcocercous cercariae

Apharyngostrigea pipientis (Faust, 1918) Olivier, 1940

The body was oval in shape. The tail was furcae, broad, and flat. There was a distinct pharynx. The esophagus reached half-way from the pharynx to acetabulum. A pair of unpigmented eye spots were anterolateral to acetabulum. There were 4 pairs of penetration glands. The excretory bladder was small. The tail was a tubule branch opening on the dorsal edge of the furcae about halfway down to the furcal tip. There were furcal finfolds (Figure 6). The following are size rangs and average sizes of 10 cercariae:

Body: 87.5-90 µm (av. 87.81 µm) x 125-175 µm(av. 140.94 µm)
Tail: 20-25 µm (av. 23.75 µm) x 150-200 µm (av. 181.25 µm)
Tail fucal: 15-20 µm (av. 19.38 µm) x 62.5-70 µm (av. 65.31 µm)
Eye spot: 3 µm x 3 µm
Oral sucker: 20-25 µm (av. 23.75 µm) x 5-35 µm (av. 29.69 µm)

Table 2. The infection rates of brackish water snails as intermediate host.

Snail	Number of examined snail	Number of infected snail		Infection rate (%)	Cercarial species
		Eastern	Central		
Assiminea brevicula	691	0	1	0.14	*Metorchis intermedius*
Cerithidea cingulata	1,068	0	1	0.09	*Heterophyes cercaria* I
Cerithidea alata	294	1	0	0.34	*Himasthla interrupta*
Cerithidea djadjariensis	623	2	0	0.32	*Ascorhytis charadriformis*
Cerithidea quadrata	105	5	0	4.76	*Hypoderaeum conoideam*
Littorinopsis intermedia	1,154	0	7	0.61	*Ascorhytis charadriformis* (4 infected snails) *Coitocaecum anaspidis* (3 infected snails)
Sermyla riqueti	918	5	1	0.54	*Haplorchis taichui* (4 snails from Eastern, 1 snails from Central) *Apharyngostiger pipientis* (1 snails from Eastern)

Ventral sucker: 12.5-22.5 µm (av. 17.92 µm) x 17.5-25 µm (av. 22.92 µm)
Pharynx: 12.5-17.5 µm (av. 15.36 µm) x 12.5 µm (av. 12.5 µm)
Excretory bladder: 50-62.5 µm (av. 54.69 µm) x 17.5-25 µm (av. 19.69 µm)

Type 3. Xiphidiocercariae

Ascorhytis charadriformis (Young, 1949) Ching, 1965

The body was oval in shape and white in color. There was a long stylet. Four pairs of penetration glands lined near a ventral sucker. The ventral sucker was bigger than the oral sucker. The tail was long and round. The excretory duct opened at the tail-end. The cercariae were produced within the sporocyst (Figure 7). The following are size ranges and average sizes of 10 cercariae:

Body: 38-53 µm (av. 45 µm) x 163-210 µm (av. 190 µm)
Tail: 13-15 µm (av. 13 µm) x 113-150 µm (av. 123 µm)
Oral sucker: 8 µm x 8 µm
Ventral sucker: 10-13 µm (av. 12 µm) x 13 µm
Excretory bladder: 13-20 µm (av. 15 µm) x 10-25 µm (av. 16 µm)
Stylet: 3 µm x 18-20 µm (av. 18 µm)

Type 4. Cotylomicrocercous cercariae

Coitocaecum anaspidis Hickman, 1934

The body was cylindrical in shape with a transparent body. The skin was covered with smaller spines. There was a stylet with double points at the oral sucker. The pre-pharynx was moderately long. The esophagus and intestine did not differ. There were 3 pairs of penetration glands. The excretory bladder was large with thick wall. The tail was cup-shape. There was an adhesive organ at the tail end (Figure 8). The cercariae were produced within the sporocyst. The following are size ranges and average sizes of 10 cercariae:

Body: 60-125 µm (av. 86 µm) x 190-313 µm (av. 230 µm)
Tail: 15-50 µm (av. 15 µm) x 43-50 µm (av. 46 µm)
Oral sucker: 35-50 µm (av. 43 µm) x 38-45 µm (av. 41 µm)
Ventral sucker: 38-50 µm (av. 46 µm) x 40-58 µm (av. 48 µm)
Excretory bladder: 30-58 µm (av. 49 µm) x 10-75 µm (av. 50 µm)
Stylet: 5-8 µm (av. 7 µm) x 8-15 µm (av. 11 µm)

Type 5. Echinostome cercariae

Himasthla interrupta Loos-Frank, 1967

The body was cylindrical in shape and colorless. The skin was covered with spines and sensory hairs. There were collar spines around the oral sucker. There was a long esophagus. The intestines were bifurcated at the ventral sucker, and ending blindly near posterior. The excretory organs were distinct and branching from the anterior to posterior of the body. The excretory bladder was a small thick wall. Both the oral and ventral suckers were large, but the ventral sucker was bigger than the oral sucker. The tail was shorter than the body. Two excretory pores at the lateral sides were about one-fourth of the tail length from its base. The cercariae were produced within the daughter redia (Figure 9). The following are size ranges and average sizes of 10 cercariae:

Body: 150-163 µm (av. 151 µm) x 243-325 µm (av. 270 µm)

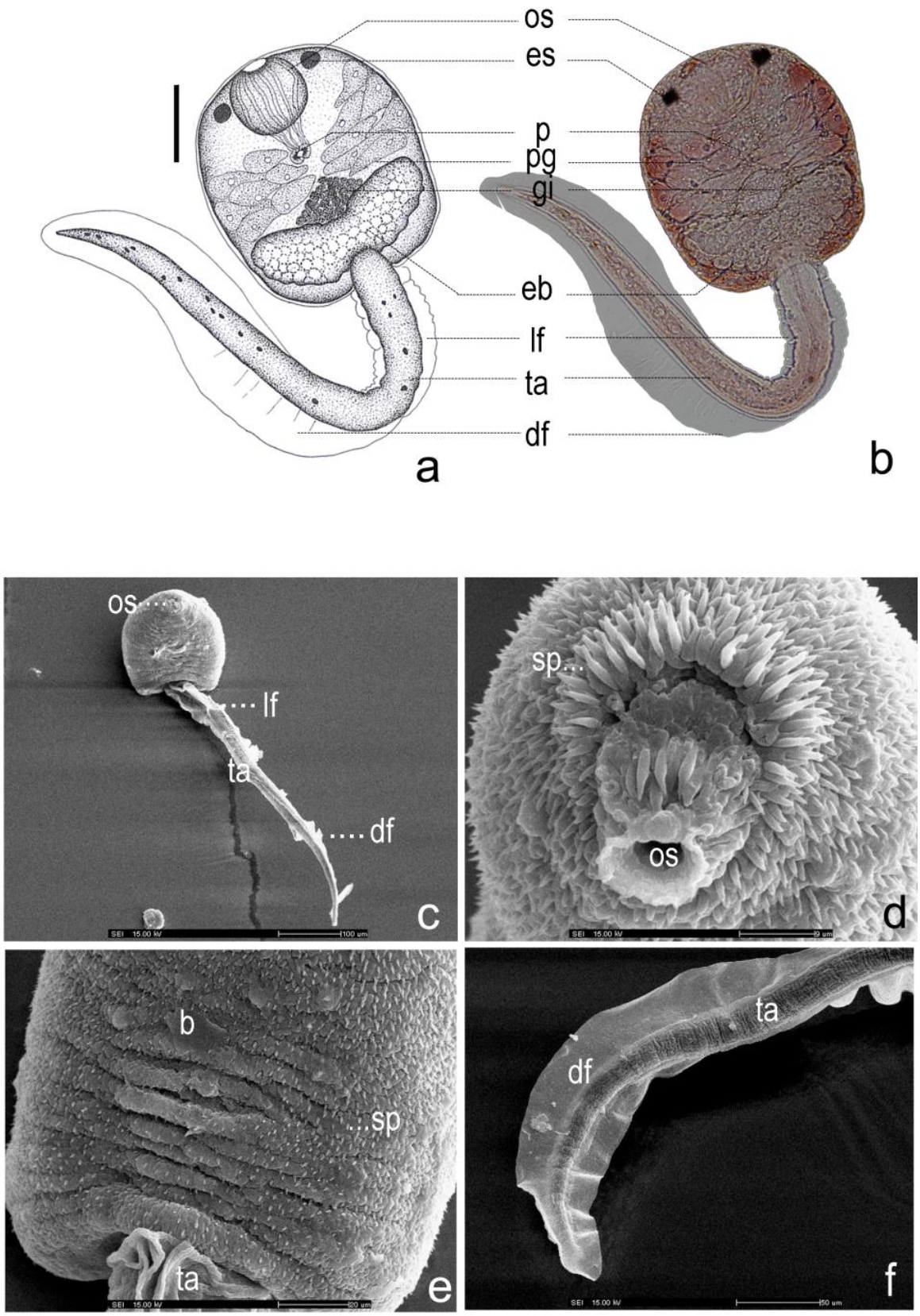

Figure 3. *Metorchis intermedius* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, (c-f). SEM micrograph of cercaria. (os = oral sucker, es = eye spot, p = pharynx, pg = penetration gland, lf = lateral finfold, df = dorso-ventral finfold, b = body) (scale bar = 100 µm).

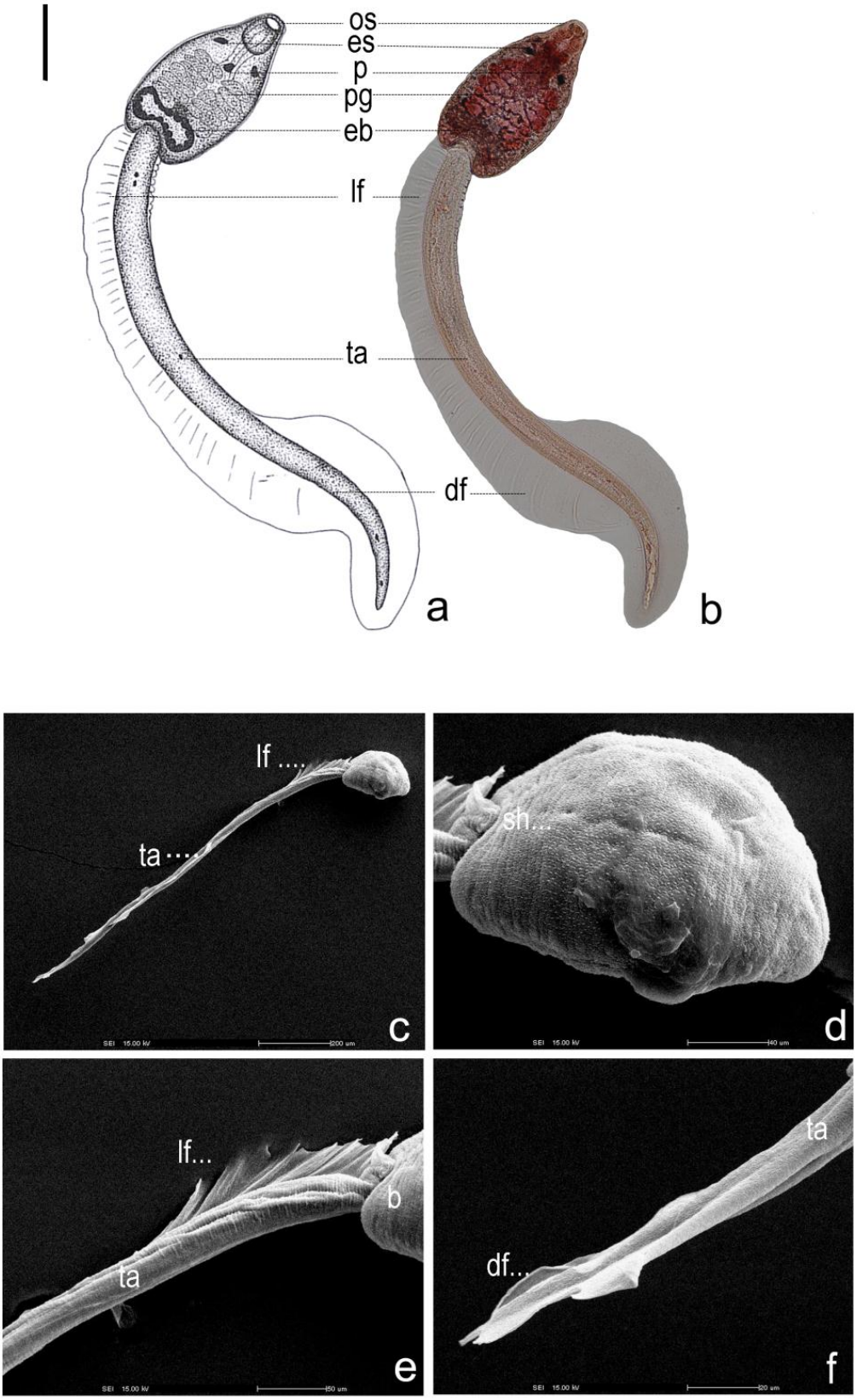

Figure 4. *Heterophyes cercaria* l cercaria: a. drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, c-f. SEM micrograph of cercaria. (os = oral sucker, es = eye spot, p = pharynx, pg = penetration gland, eb = excretory bladder, ta = tail, lf = lateral finfold, df = dorso-ventral finfold, b = body, sh = sensory hair) (scale bar = 100 µm).

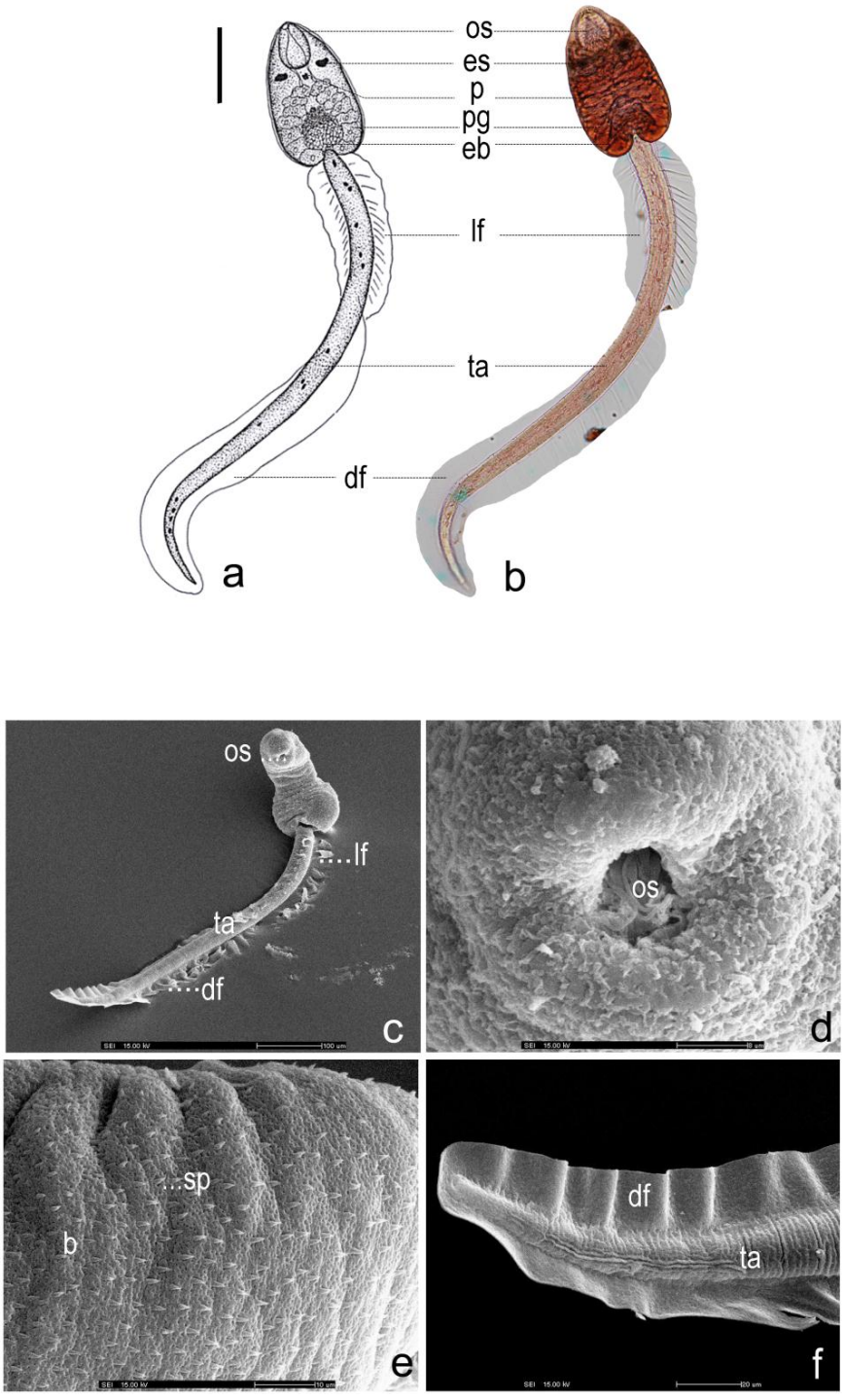

Figure 5. *Haplochis taichui* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, (c-f). SEM micrograph of cercaria. (os = oral sucker, es = eye spot, p = pharynx, pg = penetration gland, eb = excretory bladder, sp = spine, ta = tail, b = body, lf = lateral finfold, df = dorso-ventral finfold) (scale bar = 100 µm).

Tail: 28-40 µm (av. 34 µm) x 195-313 µm (av. 240 µm)
Oral sucker: 38-48 µm (av. 44 µm) x 38-48 µm (av. 44 µm)
Ventral sucker: 40-73 µm (av. 62 µm) x 55-63 µm (av. 60 µm)
Pharynx: 13-18 µm (av. 14 µm) x 20-30 µm (av. 24 µm)

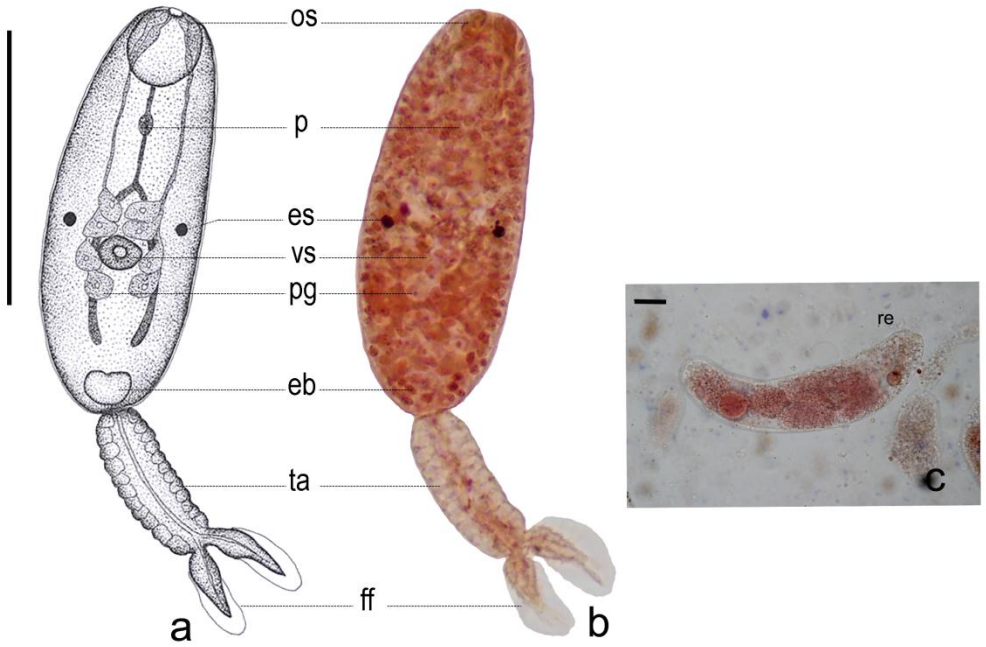

Figure 6. *Apharyngostiger pipientis* cercaria: a. drawing of cercaria stucture, (b-c). cercaria and redia stained with 0.5% neutral red. (os = oral sucker, es = eye spot, p = pharynx, pg = penetration gland, vs = ventral sucker, eb = excretory bladder, ta = tail, ff = furcal finfold, re = redia) (scale bar = 100 µm).

Excretory bladder:18-55 µm (av. 38 µm) x 18-55 µm (av. 33 µm)

Hypoderaeum conoideam (Bloch, 1982) Dietz, 1909

The body was round and colorless. There was no eye spot. There were two rows of collar spines. The first row had 15-20 spines, and the second row had 25-30 spines. There was a round pharynx. The esophagus was long; the bifurcate ceca terminated at the posterior end of the body. The ventral sucker was about two-third of the body length. The excretory vesicle was a simple sac. There was a Y-shaped excretory tube at the tail; the tube opened at the tail lateral, around one-fourth of the tail length from its base. The cercariae were produced within the rediae (Figure 10). The following are size ranges and average sizes of 10 cercariae:

Body: 270-350 µm (av. 323 µm) x 470-530 µm (av. 494 µm)
Tail: 70-90 µm (av. 79 µm) x 950-1180 µm (av. 1100 µm)
Oral sucker: 70-80 µm (av. 73 µm) x 50-70 µm (av. 65 µm)
Ventral sucker: 60-100 µm (av. 84 µm) x 60-80 µm (av. 74 µm)
Pharynx: 40-50 µm (av. 42 µm) x 40-50 µm (av. 43 µm)
Excretory bladder: 70-100 µm (av. 81 µm) x 40-60 µm (av. 50 µm)

DISCUSSION

The collected snails were separated into brackish and fresh water snails. They were collected from the river estuary and mangrove forests. Some fresh water snails found in this study are, for example, *Melanoides tuberculata*, *Tarebia granifera* and *Filopaludina martensi*. Normally, the fresh water snails were reported as the intermediate host of several trematodes, but we did not find trematode infections in this study. However, the present study confirmed the medical and veterinary importance of brackish water snails recorded in previous studies (Taraschewski & Paperna, 1981; Marorelli et al., 2006; Sri-aroon et al., 2010). In this study, either more species of snail or more species of trematodes were reported.

In terms of the species diversity of snails, we compared the species diversity of snails from the Eastern Coast and the Central Coast. We found that the Eastern Coast was more species diverse. Of the total thirty-two species found, six species of snails dispersed in the East Coast, the Central Coast, and the South of the Gulf of Thailand. These were *Assiminea brevicula*, *Littorinopsis intermedia*, *Dostia violacea*, *Cerithidea cingulata*, *Sermyla riqueti* and *Tarebia granifera*. They were common species generally found in the Gulf of Thailand (Sritongtae et al., 2015). More species of the Potamididae and Ellobiidae snails were found than others. Particularly, the family Potamididae were the most variation of snails found

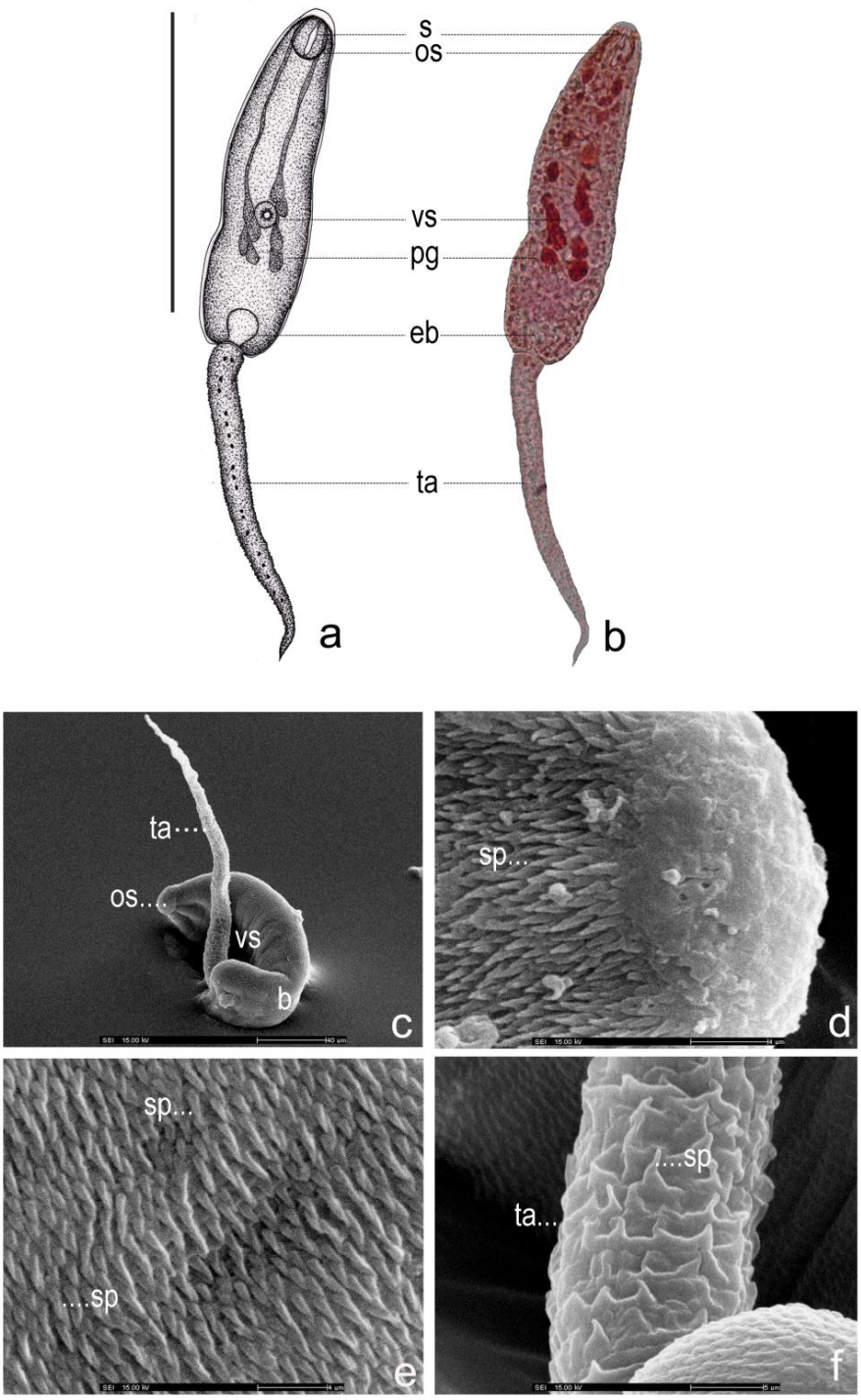

Figure 7. *Ascorhytis charadriformis* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, (c-f). SEM micrograph of cercaria. (s = stylet, os = oral sucker, vs = ventral sucker, pg = penetration gland, eb = excretory bladder, b = body, ta = tail, sp = spine) (scale bar = 100 µm).

infected with trematodes, as previously reported (Sri-aroon et al., 2010; Sritongtae et al., 2015). *C. cingulata, C. djadjariensis* and *C. quadrata* were known to be a host of human, bird and amphibian digenea. But this study showed that *C. alata* could be the intermediate host of bird trematode as well. Moreover, *Littorinopsis intermedia* was not reported with trematode infection in the past. Only *L. scabra* was mentioned. But only in this study *L.*

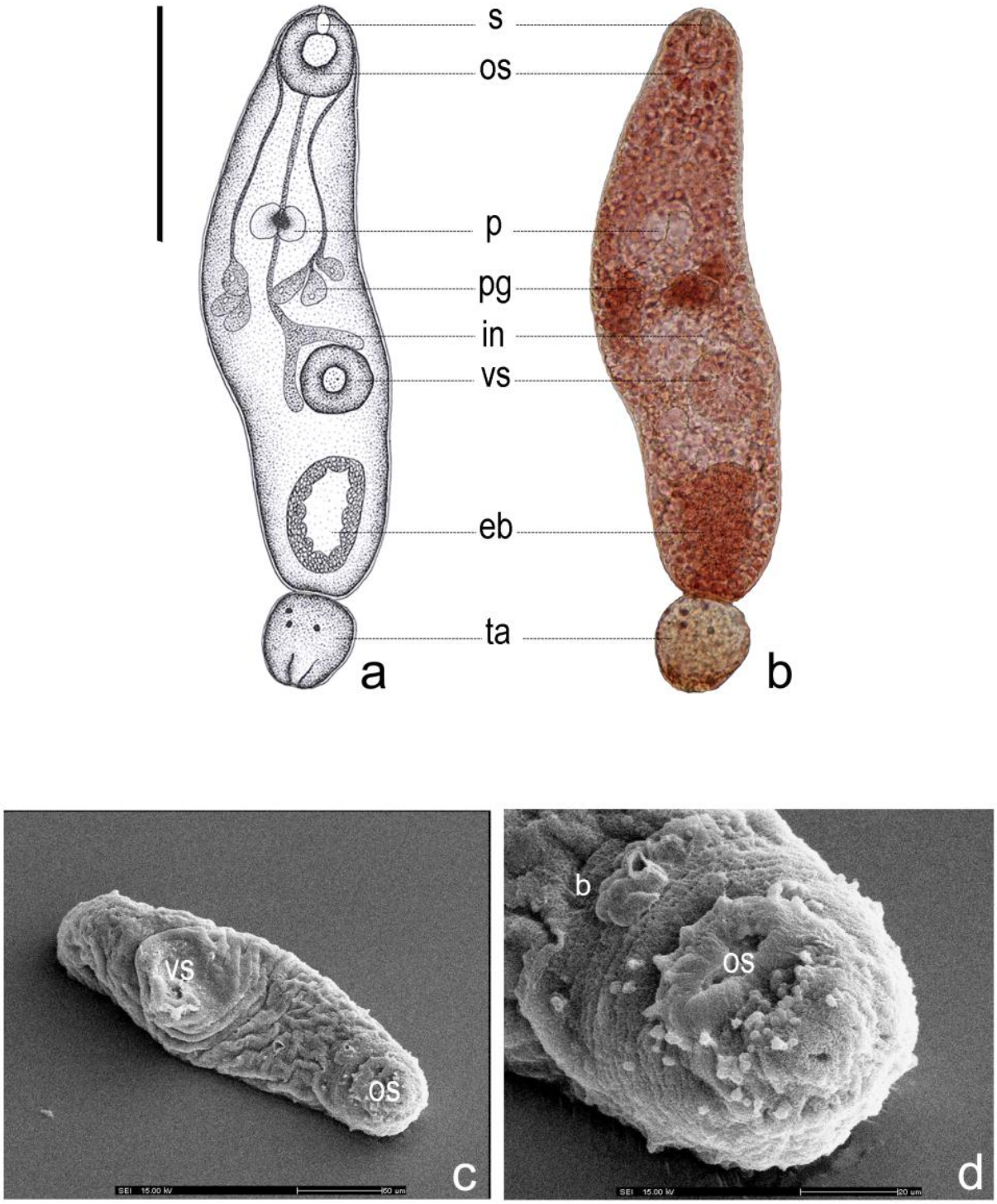

Figure 8. *Coitocaecum anaspidis* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, c-d. SEM micrograph of cercaria. (s= stylet, os = oral sucker, p = pharynx, vs = ventral sucker, pg = penetration gland, in = intestine, eb = excretory bladder, ta = tail, b = body) (scale bar = 100 µm).

intermedia was found infected with *Ascorhytis charadriformis* (amphibian trematode), while *L. scabra* was not. Thiarid snails, *Sermyla riqueti* were infected with *Apharyngostiger pipentis* (fish trematode) and *Haplorchis taichui* (human minute intestinal fluke), causing a major public health problem in Thailand and the other countries

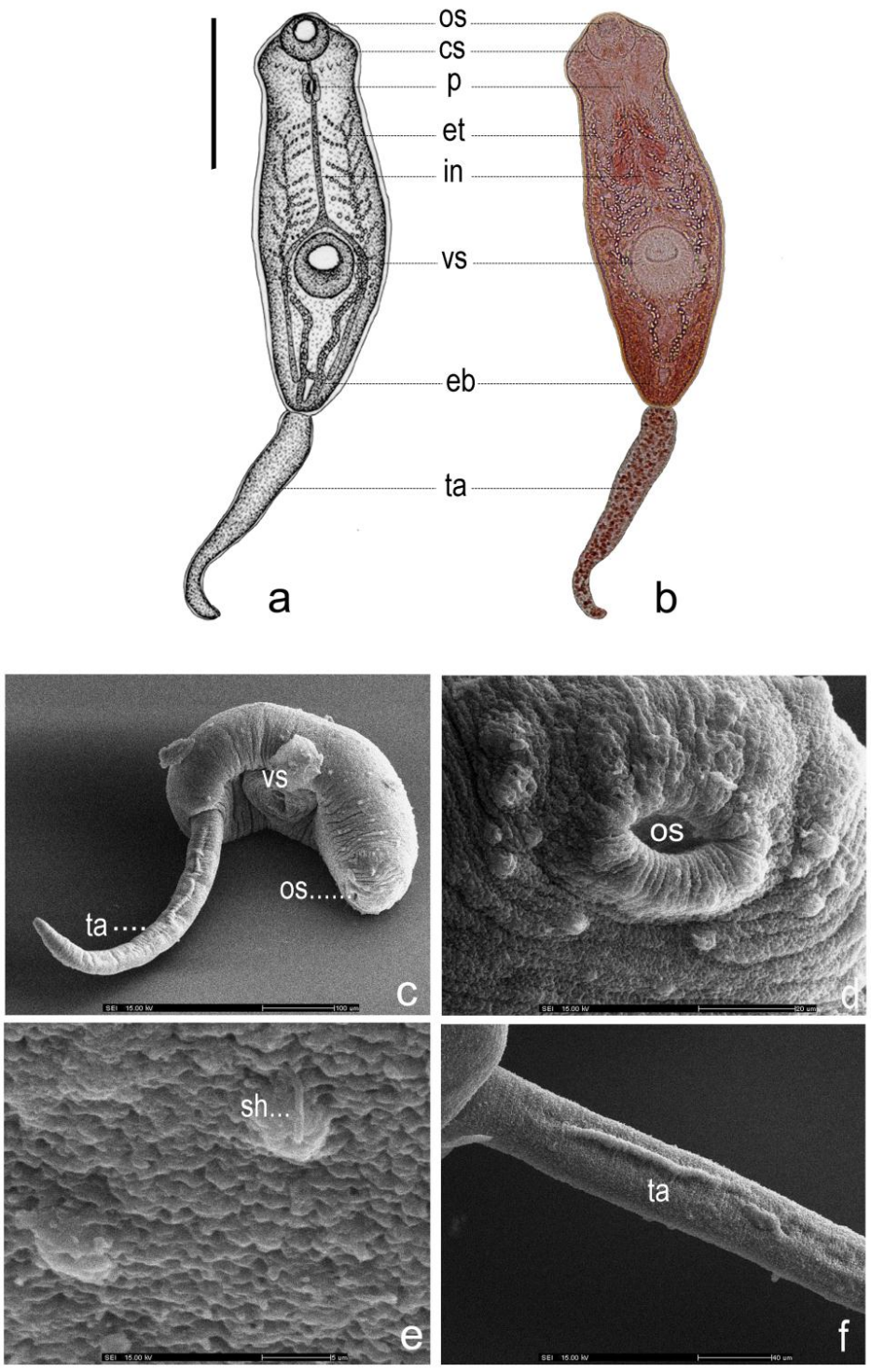

Figure 9. *Himasthla interrupta* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, c-f. SEM micrograph of cercaria. (os = oral sucker, cs = collar spines, p = pharynx, in = intestine, vs = ventral sucker, eb = excretory bladder, et = excretory tube, ta = tail, sh = sensory hair) (scale bar = 100 µm).

in South East Asia (Chai et al., 2005; Krailas et al, 2011). *S. riqueti* was reported with the highest infection rate in a previous study of cercarial infections of brackish water snails on the east coast of southern Thailand (Sritongtae

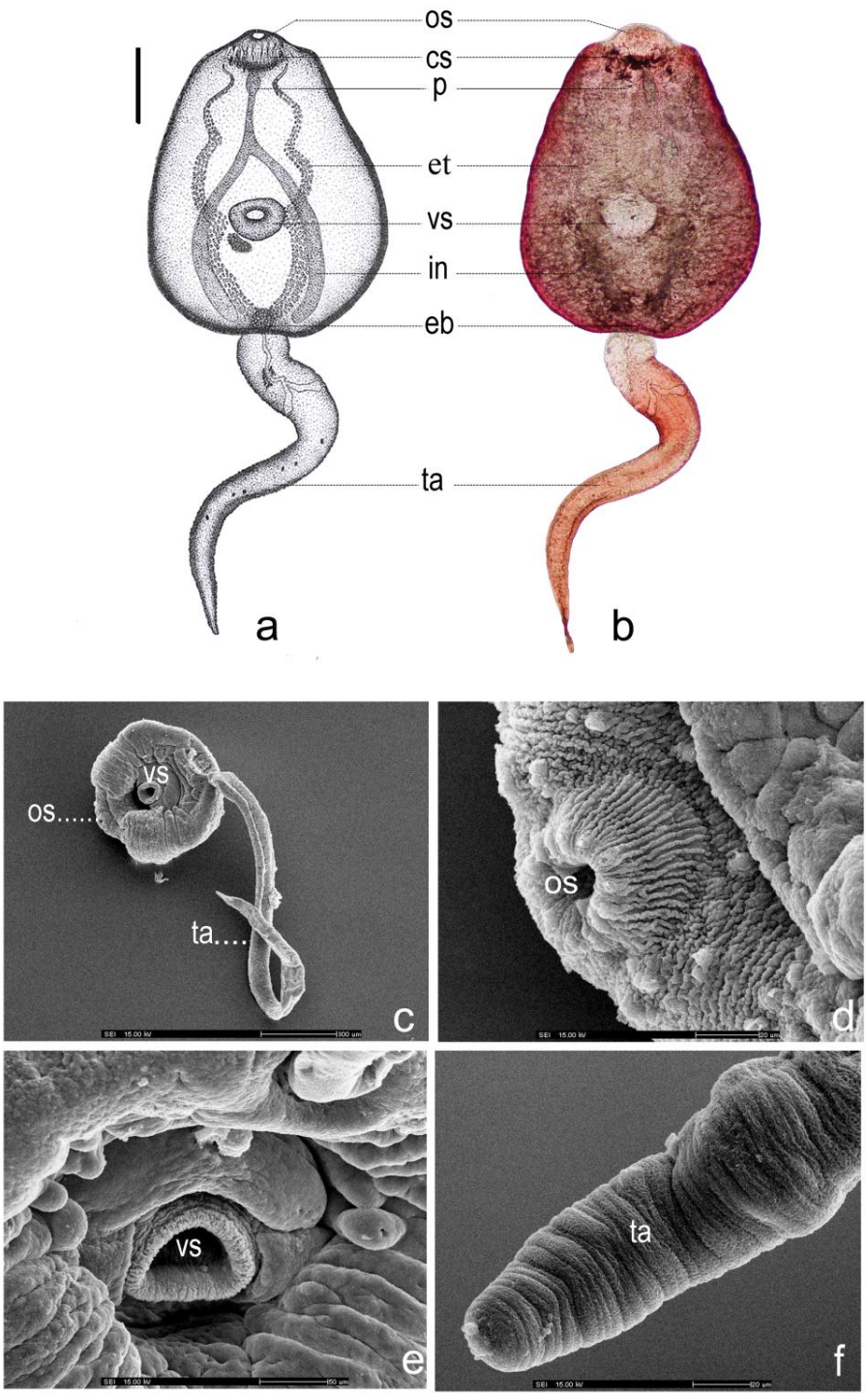

Figure 10. *Hypoderaeum conoideam* cercaria: a. drawing of cercaria stucture, b. cercaria stained with 0.5% neutral redia, c-f. SEM micrograph of cercaria. (os = oral sucker, cs = collar spines, p = pharynx, in = intestine, vs = ventral sucker, eb = excretory bladder, et = excretory tube, ta = tail) (scale bar = 100 µm).

et al., 2015). In the present study, *C. quadrata* had the highest infection rate with *Hypoderaeum conoideam* (human echinostome fluke).It should also be noted here that identification into groups might not be sufficient. This study showed that identification at the species level was very useful to determine the exact species of trematodes

and their intermediate hosts.

Conflict of Interest

The authors have not declared any conflict of interest.

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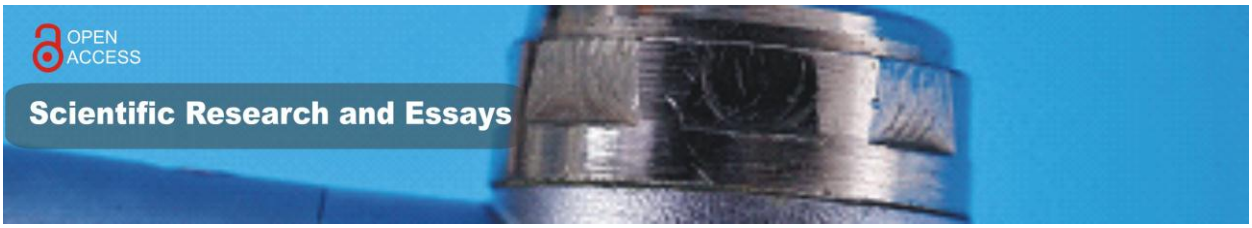

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